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Drinking Water Criteria Document for Heptachlor Heptachlor Epoxide and Chlordane

Syracuse Fesearch Corp., NY

Prepared for:

Environmental Protection Agency, Cincinnati, OH

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DRINKING WATER CRITERIA DOCUMENT FOR HEPTACHLOR, HEPTACHLOR EPOXIDE AND CHLORDANE

Prepared for

OFFICE OF DRINKING WATER

Prepared by

Environmental Criteria and Assessment Office
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16. ABSTRACT

This document deals with the drinking water health effects of heptachlor, heptachlor epoxide & chlordane through the review of several studies. These studies include animal and humans. Physical and chemical properties are discussed. Carcinogenicity of the compound is reviewed and evaluated through various studies. Any existing guidelines and standards are presented.

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1984; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADP	Adenosine diphosphate
AEL	Adverse effect level
cyclic AMP	Adenosine 3':5'-cyclic phosphate
ATPase	Adenosine triphosphatase
CCl_4	Carbon tetrachloride
CI	Confidence interval
CNS	Central nervous system
DWEL	Drinking water equivalent level
EEG	Electroencephalogram
FEL	Frank effect level
GC-MS	Gas chromatography-mass spectrometry
GI	Gastrointestinal
GLC	Gas liquid chromatography
GOT	Glutamine oxalacetic transaminase
GPT	Glutamic pyruvic transaminase
HA	Health advisory
i.p.	Intraperitoneal
i.v.	Intravenous
LOAEL	Lowest-observed-adverse-effect level
MW	Molecular weight
NADH	Reduced nicotinamide-adenine dinucleotide
NADPH	Reduced nicotinamide-adenine dinucleotide phosphate
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level

LIST OF ABBREVIATIONS (cont.)

ppm	Parts per million
RfD	Reference dose
RNA	Ribonucleic acid
SMR	Standardized mortality ratio
TLC	Thin-layer chromatography
TWA	Time-weighted average

I. SUMMARY

Chlordane (MW 409.76) and heptachlor (MW 373.32) are structurally related chlorinated hydrocarbon insecticides used to control termites and pests on field crops. Heptachlor epoxide (MW 389.32) is an oxidation product of heptachlor. Technical grade chlordane consists of approximately equal parts cis- and trans- isomers, along with heptachlor and other impurities, and has a low water solubility. Technical grade heptachlor consists of ~73% heptachlor and 22% trans-chlordane, and also has a low water solubility. Heptachlor epoxide is slightly water soluble.

Neither chlordane nor heptachlor epoxide undergoes significant chemical reactions in drinking water; however, both may volatilize during aeration of drinking water. Chlordane, heptachlor and heptachlor epoxide may sorb to organic particulate matter and be removed from drinking water during filtration. Heptachlor may hydrolyze in drinking water and may undergo oxidation with singlet oxygen during the water treatment process. Heptachlor will probably volatilize faster than chlordane and heptachlor epoxide during aeration.

The absorption of chlordane by the gastrointestinal tract of animals can be inferred from data on its systemic toxicity and from excretion data. Rats absorbed at least 2-8%, while rabbits absorbed at least 30% of orally administered chlordane. Humans also absorbed chlordane after accidental poisoning. Chlordane and/or metabolites are rapidly distributed to tissues of animals, with highest levels detected in adipose tissue where it persists as the metabolite oxychlordane. Oxychlordane has been detected in human adipose tissue. At least 13 or 14 fecal metabolites and a urinary glucuronide conjugate have been isolated in rats. Metabolism involves dehydrogenation, epoxidation (to oxychlordane), hydroxylation and dechlorination reactions. Metabolism by human microsomes in vitro is similar to metabolism by rat microsomes in vitro. The major route of excretion of chlordane and metabolites in rats is via the feces with 50% of a single oral dose excreted in 10 days. Rabbits eliminate more chlordane metabolites in the urine than in the feces. In humans, the half-time for elimination from serum has been measured at 21-88 days. Oxychlordane has been found in the milk of lactating women.

Heptachlor is absorbed from the gastrointestinal tract of rats; residues appear in blood within an hour of oral dosing. Absorption of heptachlor by humans can be inferred from reports of heptachlor epoxide levels in adipose tissue of the general population. Heptachlor epoxide, the major metabolite of heptachlor, is distributed to tissues of animals; the highest levels are detected in adipose tissue where it persists. Heptachlor is epoxidized to heptachlor epoxide that is dechlorinated and hydroxylated to a metabolite eliminated in the feces. Other routes of metabolism involve epoxidation of other intermediates. Rat liver microsomes form 4 times more heptachlor epoxide than human microsomes; otherwise, in vitro metabolism is similar. Rats eliminate >50% of administered heptachlor as fecal metabolites; <5% is eliminated in urine.

The acute oral LD_{50} of chlordane (in a lipophilic vehicle) is ~350 mg/kg bw in rats, 390 mg/kg bw in mice and 1720 mg/kg bw in hamsters. Neonatal rats are less sensitive to chlordane toxicity than are adults.

Symptoms of acute intoxication include CNS disturbances, such as irritability, tremors and convulsions. Sublethal doses result in microsomal enzyme induction and enhanced gluconeogenesis. Subchronic dietary administration of chlordane to rats and mice has resulted in changes in prostate homeostasis and microsomal enzyme induction. The no-effect level for enzyme induction is <5 mg/kg diet. In chronic dietary studies, a no-effect level of chlordane in rats for histopathologic changes in liver, kidney, lungs, pancreas, stomach, adrenals, thyroid, thymus, lymph, testes, ovaries, heart and spleen; for food consumption; for growth rate; and for mortality was 5 mg/kg diet. At 10 mg/kg diet, mild liver changes (e.g., hepatocytomegaly) were observed. At 150-300 mg/kg, progressively more severe histopathologic damage to liver and other organs occurred.

The published literature on mutagenicity testing of chlordane and heptachlor/heptachlor epoxide is quite similar; indeed, most studies report results on both chemicals. Generally, the results have indicated that these chemicals are not mutagenic in bacteria or in mammalian cells in culture and do not induce DNA repair as measured by unscheduled DNA synthesis in rodent hepatocytes. While dominant lethal tests in mice have been negative for both chemicals, the absence of direct cytogenetics tests in both germinal and somatic cells precludes a conclusion of their potential for causing chromosome aberrations.

Heptachlor was more toxic than chlordane to laboratory animals. The acute oral LD_{50} of heptachlor is ~100 mg/kg bw for rats, 70 mg/kg bw for mice and 105 mg/kg bw for hamsters. Neonatal rats were less sensitive than adult rats to heptachlor administered intraperitoneally. The acute LD_{50}

of heptachlor epoxide in rats is ~60 mg/kg bw. Symptoms of acute intoxication of heptachlor and heptachlor epoxide included tremors, convulsions, paralysis and hypothermia. Sublethal dietary doses resulted in microsomal enzyme induction; the no-effect level was <2 mg/kg diet. Moderate liver damage in rats was accompanied by increased serum levels of hepatic enzymes at dietary levels of 7 or 12 mg/kg bw/day for 14 days; by 28 days tolerance appeared to develop.

Subchronic dietary exposure to heptachlor has been associated with changes in rat prostate homeostasis and induction of hepatic microsomal enzymes. Chronic dietary exposure to heptachlor or heptachlor epoxide has resulted in hepatocytomegaly, hyperplasia, hepatic vein thrombosis and cirrhosis in mice.

For humans, clinical case studies of acute exposure to chlordane describe CNS disturbances. An estimated dose of 0.14 mg/kg bw chlordane resulted in convulsions and seizures in a child. Death occurred in a woman who ingested 104 mg chlordane/kg bw. Blood dyscrasias, such as aplastic anemia, leukemia and pancytopenia, have been described in cases involving exposure to chlordane and heptachlor, either alone or in combination with other pesticides. Neuroblastoma has been associated with pre- and/or postnatal exposure to chlordane and heptachlor.

Based on the accumulated evidence, chlordane is a probable human carcinogen, classified in Group B2 under the U.S. EPA's guidelines for carcinogen risk assessment. Animal studies provide sufficient evidence for carcinogenicity: chlordane increased the incidence of liver carcinomas in C57Bl/6N, CD-1 and B6C3F1 mice; liver adenomas and hemangiomas in ICR mice;

and liver adenomas in Fischer 344 rats. Epidemiologic studies provide inadequate evidence for carcinogenicity due to methodology and data limitations.

Heptachlor/heptachlor epoxide is a probable human carcinogen, classified in Group B2 under the U.S. EPA's guidelines for carcinogen risk assessment. Animal studies provide sufficient evidence for carcinogenicity: heptachlor/heptachlor epoxide increased the incidence of liver carcinomas in C3H, CD-1 and B6C3F1 mice and in CFN rats. Epidemiologic studies provide inadequate evidence for carcinogenicity due to methodology and data limitations.

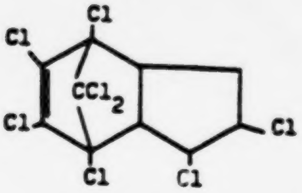
Several studies on the mechanism of neurotoxicity indicate that EEG changes, acetylcholine content of brain, ammonia and glutamate content of brain, and inhibition of synaptosomal ATPases are involved in CNS toxicity. Other mechanisms studied included enhanced gluconeogenesis and effects on cellular respiration. Phenobarbital and turpentine enhanced the toxicity of chlordane and heptachlor in rats. Chlordane pretreatment enhanced the toxicity of CCl_4. The content and quality of protein in the diet affects the toxicity of chlordane and heptachlor. An epigenetic mechanism for carcinogenicity has been suggested.

Quantitative risk assessment for chlordane was based on animal data since human data were insufficient. Satisfactory dose-response data was not available for a derivation of a 1-day HA for chlordane. A 10-day HA for chlordane for a 10 kg child is 0.063 mg/ℓ. A DWEL derived from chlordane toxicity data is 0.002 mg/ℓ. The concentrations of chlordane in water corresponding to an increased upper limit lifetime risk of cancer of 10^{-4}, 10^{-5} and 10^{-6} are 2.7, 0.27 and 0.027 μg/ℓ, respectively.

Quantitative risk assessment for heptachlor and heptachlor epoxide was based on animal toxicity data, since human data were insufficient. A 1-day HA for heptachlor for a 10 kg child is 10 µg/ℓ. Because there was a paucity of data to derive a 10-day HA and because there was some indication that rats develop tolerance to heptachlor, the value for a 1-day HA was recommended to serve as the 10-day HA, as well. A DWEL derived from heptachlor toxicity data is 20 µg/ℓ. A DWEL derived from heptachlor epoxide toxicity data is 0.4 µg/ℓ. The concentrations of heptachlor in drinking water corresponding to increased lifetime risk of cancer of 10^{-4}, 10^{-5} and 10^{-6} are 0.76, 0.076 and 0.0076 µg/ℓ, respectively. The concentrations of heptachlor epoxide in drinking water corresponding to increased lifetime risk of cancer of 10^{-4}, 10^{-5} and 10^{-6} are 0.38, 0.038 and 0.0038 µg/ℓ, respectively.

II. PHYSICAL AND CHEMICAL PROPERTIES

Chlordane



Molecular formula: $C_{10}H_6Cl_8$

Molecular weight: 409.76

RTECS number: PB 98000

Chemical Abstracts Service number: 57-74-9 and 12789-03-6

Chlordane is the common name for 1,2,4,5,6,7,8,8-octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene. This chemical is also known as dichlorodene; 1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindane; and 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanindan, among others. Some trade names of chlordane include Octachlor, Velsicol 1068, Toxichlor and Dowchlor (IARC, 1979a).

Chlordane is a contact insecticide (BCPC, 1977) and has been used in the control of cutworms, ants root weevils, rose beetles, grasshoppers, grubs and termites (Deichmann, 1981). Technical grade chlordane consists of 24% transchlordane, 21.5% chlordene isomers, 19% cis-chlordane (CAS No. 5103-74-2; RTECS No. PC01750), 10% heptachlor, 7% nonachlor and 18.5% other miscellaneous impurities (IARC, 1979a). HCS-3260, a high purity formulation (98+%) contains 3:1 cis/trans chlordanes.

The chemical and physical properties of chlordane, a viscous amber liquid (IARC, 1979a), are given in Table II-1.

The fate of chlordane in aquatic media cannot be assessed with certainty. Although there is evidence that chlordane may undergo sensitized (acetone, etc.) photolysis in aquatic media (Callahan et al., 1979), the direct photolysis of this compound in ambient aquatic media without any sensitizer appears to be an insignificant process (Verschueren, 1983; Mabey et al., 1981). From the oxidation rate constant values given by Mabey et al. (1981), the two likely oxidation processes in aquatic media, involving singlet oxygen and peroxy/radical, do not appear to be significant processes for chlordane. Similarly, both hydrolysis and biodegradation of this compound in ambient aquatic media are not likely to be significant fate-determining processes (Mabey et al., 1981; Callahan et al., 1979; Tabak et al., 1981). The three likely processes that may determine the fate of this compound in aquatic media are evaporation, sorption and bioaccumulation. The evaporative half-life of this compound from 1 m depth of water was estimated to be 28-33 hours (Atlas et al., 1982). Thus, although the evaporation from aquatic media may not be rapid, it could be significant in the absence of any other faster processes.

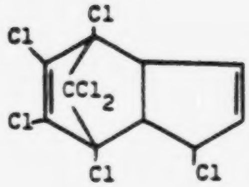
From the sediment-water sorption coefficient value (1.4×10^{-5}) given by Mabey et al. (1981), chlordane is expected to be significantly sorbed from the aquatic phase to suspended particles and sediment of high organic content in water. Therefore, sorption may play a significant role in determining the aquatic fate of chlordane (Callahan et al., 1979). In the treatment of wastewater, a 2.1 mg carbon/ℓ water was required to reduce the pollutant concentration from 0.1 to 0.01 mg/ℓ (U.S. EPA, 1981).

TABLE II-1

Chemical and Physical Properties of Chlordane

Property	Value or Comment	Reference
Boiling point	175°C at 2 mm Hg	Callahan et al., 1979
Melting point	106-107°C (cis-isomer) 104-105°C (trans-isomer)	BCPC, 1977
Specific gravity	1.59-1.63 at 25°C	Deichmann, 1981
Vapor pressure	1 x 10^{-5} mm Hg at 25°C	IARC, 1979a
Solubility	9 µg/ℓ at 25°C in water for technical grade and 56 µg/ℓ in water for cis:trans (75:25) chlordane; miscible with aliphatic and aromatic hydrocarbon solvents	Verschueren, 1983; Deichmann, 1981
Evaporation half-life ($t_{1/2}$) from water	28 hours (trans-isomer) from 1 meter depth; 33 hours (cis-isomer) from 1 meter depth	Atlas et al., 1982
Log (octanol/water partition coefficient)	3.32	Rao and Davidson, 1982
Air conversion factor	1 mg/ℓ = 59.7 ppm and 1 ppm = 16.76 mg/m^3 at 25°C; 760 mm Hg	Deichmann, 1981

Heptachlor



Molecular formula: $C_{10}H_5Cl_7$

Molecular weight: 373.32

RTECS number: PC 07000

Chemical Abstracts Service number: 76-44-8

Heptachlor is the common name for 1,4,5,6,7,8,8-heptachlor-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene. This chemical is also known as 3-chlorochlordene and 3,4,5,6,7,8,8a-heptachlorodicyclopentadiene. Some trade names of heptachlor include Velsicol 104, Drinox, Heptagran, Rhodiachlor and E3314 (IARC, 1979b).

Heptachlor is a contact insecticide with some fumigant action (BCPC, 1977) used to control termites and insects that attack field crops (Deichmann, 1981). Technical heptachlor consists of ~73% heptachlor, 22% trans-chlordane and 5% nonachlor (Deichmann, 1981).

The chemical and physical properties of 99% pure heptachlor (unless otherwise stated), a white crystalline solid (IARC, 1979b), are given in Table II-2.

TABLE II-2

Chemical and Physical Properties of Heptachlor

Property	Value or Description	Reference
Boiling point	135-145°C at 1-1.5 mm Hg	IARC, 1979b
Melting point	93°C	IARC, 1979b
Specific gravity	1.57-1.59 (unknown purity)	Deichmann, 1981
Vapor pressure	3×10^{-4} mm Hg at 25°C	IARC, 1979b
Absorption spectrometry	λ_{max} 236 nm $(E^{1}_{1} = 1611)$, 309 nm $(E = 270)$, 328 nm $(E^{1}_{1} = 203$ in ethanol)	IARC, 1979b
Log (octanol/water partition coefficient)	3.87 (measured) 5.44*	Rao and Davidson, 1982 Mackay, 1982
Solubility	in water, 0.056 mg/ℓ at 25°C; soluble in many organic solvents	BCPC, 1977; IARC, 1979b
Air conversion factor	1 mg/ℓ = 65.1 ppm and 1 ppm = 15.35 mg/m³ at 25°C, 760 mm Hg	Deichmann, 1981

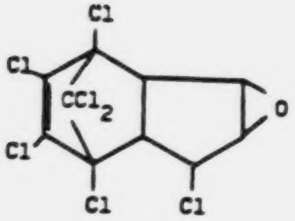
*Estimated from reverse phase HPLC data

The fate of heptachlor in aquatic media has been studied by several investigators. This compound may undergo significant photolysis in ambient aquatic media (Callahan et al., 1979). Verschueren (1983) reported that sunlight and artificial fluorescent light irradiation of 10 μg/ℓ of this compound in a river water placed inside a sealed glass jar produced 75% decomposition in 1 week and complete decomposition in 2 weeks. From the estimated oxidation rate constant values given by Mabey et al. (1981), the singlet oxygen reaction with heptachlor in aquatic media may be significant. Based on an estimated rate constant of 3×10^{-10} M^{-1} hr^{-1} (Mabey et al., 1981), and a concentration of 10^{-12} M for singlet oxygen in surface water (Mill et al., 1982), the half-life of heptachlor for this reaction is estimated to be ~1 day.

The hydrolysis of heptachlor in aquatic media is also an important process. It has been estimated that the hydrolytic half-life of heptachlor is in the range of 1-3 days (Callahan et al., 1979; Mabey et al., 1981). The volatilization half-life of heptachlor from aquatic media is estimated to be in the range of 2-10 days from pond, river and lake water. This estimate is based on the re-aeration rate ratio of 0.355 given by Mabey et al. (1981) and oxygen re-aeration rate of 0.19 day^{-1}, 0.96 day^{-1} and 0.24 day^{-1} for pond, river and lake water, respectively as given by Mill et al. (1982). Although the evaporative half-life of heptachlor in aquatic media appears to be longer than that of chlordane (2-10 days vs. 28-33 hours) from this estimate, Huang (1970) reported that the evaporation rate of heptachlor from aquatic media is faster than chlordane.

From the sediment-water sorption coefficient value of 1.2×10^{-4} given by Mabey et al. (1981), heptachlor may be significantly sorbed onto suspended particles and sediments of high organic content present in aquatic media. Therefore, sorption may play a significant role in determining the fate of heptachlor in aquatic media (Callahan et al., 1979). In the treatment of wastewater, 5.9 mg carbon/ℓ water was required to reduce the pollutant concentration from 0.1 to 0.01 mg/ℓ (U.S. EPA, 1981). The biodegradation rate of heptachlor in aquatic media is slower than the rate of hydrolysis (Mabey et al., 1981; Callahan et al., 1979).

Heptachlor Epoxide



Molecular formula: $C_{10}H_5Cl_7O$

Molecular weight: 389.32

RTECS number: PB 94500

Chemical Abstracts Service number: 1024-57-3

Heptachlor epoxide is the common name for 2,3,4,5,6,7,7-heptachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2H-indene(1,2-b)oxirene. This chemical is also known as epoxyheptachlor; Velsicol 53-CS-17; ENT 25, 584; 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan. Heptachlor epoxide is not commercially available in the United States, but it is an oxidation product of heptachlor (IARC, 1979b; Verschueren, 1983).

The chemical and physical properties of heptachlor epoxide are given in Table II-3.

The fate of heptachlor epoxide in aquatic media has not been comprehensively studied. From the information available in the reviewed literature, photolysis, oxidation or hydrolysis are not expected to be a significant fate-determining process for this chemical in aquatic media (Mabey et al., 1981; Callahan et al., 1979; Verschueren, 1983). From the estimated biotransformation rate constant (3×10^{-9} mℓ $cell^{-1}$ hr^{-1}) given by Mabey et al. (1981), and the estimated microbial population in surface water (5×10^{-5} cell $m\ell^{-1}$) given by Burns et al. (1981), the biodegradation of this compound does not appear to be a significant process.

The two processes that may determine the fate of heptachlor epoxide in aquatic media are volatilization and sorption. Huang (1970) reported that the rate of volatilization of heptachlor epoxide from aquatic media is much slower compared with heptachlor. However, it is difficult to make a reasonable estimate of the evaporative half-life for this compound because of the lack of appropriate data. The sorption of this compound on suspended particulate matter or sediment of high organic content may be a moderately significant process (Mabey et al., 1981; Callahan et al., 1979). In the treatment of wastewater, a 2.2 mg/ℓ carbon dose was required to reduce the concentration of this compound from 0.1 to 0.01 mg/ℓ (U.S. EPA, 1981).

TABLE II-3

Chemical and Physical Properties of Heptachlor Epoxide

Property	Value or Description	Reference
Melting point	160-161.5°C (99.5% pure)	IARC, 1979b
Vapor pressure (estimated)	3 x 10^{-4} mm Hg at 25°C	Mabey et al., 1981
Solubility	0.35 mg/ℓ at 25°C	Mabey et al., 1981
Log octanol/water* partition coefficient	5.40 4.43 2.65	Mackay, 1982 Geyer et al., 1982 Mabey et al., 1981
Conversion factor	1 ppm = 15.9 mg/m³ at 25°C and 760 mm of Hg	

*The difference in these calculated values is due to the different methods used for estimating the partition coefficient.

Summary

Chlordane and heptachlor are structurally related chlorinated hydrocarbon insecticides that have been and/or are being used to control pests on field crops and termites. Heptachlor epoxide is a degradation product of heptachlor. Technical grade chlordane is a yellow-colored liquid consisting of approximately equal parts cis- and trans-chlordane isomers, along with chlordane isomers, heptachlor, nanachlor and their impurities. It boils at 175°C at 2 mm Hg (Callahan et al., 1979), melts at 104-107°C (BCPC, 1977), has a vapor pressure of 1×10^{-5} mm Hg at 25°C (IARC, 1979a) and has a low water solubility but is soluble in most hydrocarbon solvents (Deichmann, 1981). Chlordane has a log octanol/water partition coefficient of 3.32 (Rao and Davidson, 1982).

Technical grade heptachlor is approximately 73% heptachlor and 22% transchlordane. The boiling point of the pure chemical (a white crystalline solid) is 135-145°C at 1-1.15 mm Hg; its melting point is 93°C and its vapor pressure is 3×10^{-4} mm Hg at 25°C (IARC, 1979b). Like chlordane, it has a low water solubility but is soluble in organic solvents. Literature values for log octanol/water partition coefficient were reported as 3.87 (Rao and Davidson, 1982) and 5.44 (Mackay, 1982) for heptachlor.

The melting point of pure (99.5%) heptachlor epoxide is 160-161.5°C (IARC, 1979b) and has an estimated vapor pressure of 3×10^{-4} mm Hg at 25°C (Mabey et al., 1981). It is slightly soluble in water (Mabey et al., 1981) and has a log (octanol/water partition coefficient) in the range of 2.65-5.40 (Mackay, 1982; Geyer et al., 1982; Mabey et al., 1981).

Neither chlordane nor heptachlor epoxide will undergo significant chemical reactions in drinking water (Callahan et al., 1979; Mabey et al., 1981). Volatilization of both chlordane and heptachlor epoxide is possible during aeration of drinking water; however, the rate of volatilization is faster for chlordane than heptachlor epoxide (Huang, 1970). Both compounds may be sorbed onto organic particulate matter and may be removed during the filtration processes. The rate of sorption with chlordane is higher than heptachlor epoxide (Mabey et al., 1981; Callahan et al., 1979). Heptachlor, on the other hand, may hydrolyze in drinking water (Mabey et al., 1981; Callahan et al., 1979) and may undergo oxidation with singlet oxygen (Mabey et al., 1981) during the water treatment process. Volatilization of heptachlor is possible during aeration of drinking water at a rate faster than that of either chlordane or heptachlor epoxide (Huang, 1970). Heptachlor may be sorbed onto organic particulate matter (Mabey et al., 1981; Callahan et al., 1979) and may be removed during filtration of drinking water.

III. TOXICOKINETICS

Animal Studies

Chlordane.

Absorption -- Quantitative data on the absorption of chlordane from the gastrointestinal tract of animals are not available; however, the systemic toxicity of the insecticide to laboratory animals following oral exposure (Chapter V) indicates that some absorption does take place. That chlordane is absorbed can also be inferred from excretion data. As discussed in Chapter II, chlordane consists of a mixture of components, including the cis- and trans-isomers. A high purity (98+%) formulation of the insecticide, designated HCS-3260, contains cis- and trans-chlordane in a 3:1 ratio. Sprague-Dawley rats (one of each sex) received a single dose of 0.05, 0.2 or 1.0 mg ^{14}C-HCS-3260/kg bw by gavage, or a single oral dose of each ^{14}C-labeled isomer at 0.2 mg/kg bw (Barnett and Dorough, 1974). Elimination of radioactivity in the urine over 7 days was 6% for females and 2% for males following ^{14}C-HCS-3260 treatment. Following treatment with cis- and trans-chlordane-^{14}C, female rats eliminated 8.5 and 5% of the administered radioactivity, respectively. These results indicate that at least 2-8.5% of the administered chlordane dose was absorbed by the gastrointestinal tract of rats. In contrast, a male rabbit that was given ^{14}C-HCS-3260 in a dietary concentration of 25 ppm (25 mg/kg diet) for 2 days excreted 33% of the radioactivity in the urine and 21% in the feces 24 hours after termination of the treatment (Barnett and Dorough, 1974).

Pulmonary absorption of an unspecified amount of ^{14}C-chlordane (11,500 dpm/μg) in 20 μℓ ethanol administered as an aerosol intratracheally to female Sprague-Dawley rats was observed by Nye and Dorough (1976). No intact ^{14}C-chlordane was detected in exhaled air. A peak blood concentration of radioactivity of ~4% of the applied dose was reached in <5 minutes.

Ambrose et al. (1953b) reported in an abstract that dermal application of 50 mg chlordane/kg bw was more toxic to rats when the chemical was applied in cottonseed oil rather than ethyl alcohol, indicating greater absorption with the lipophilic vehicle.

Distribution -- The tissue distribution of ^{14}C-HCS-3260, cis- or trans-chlordane-^{14}C and the metabolite, oxychlordane, in male and female rats following treatment with single oral doses was compared by Barnett and Dorough (1974). At one day following doses of 0.05-2.0 mg/kg bw of the respective compounds, the concentrations of radioactive equivalents in brain, muscle, liver and kidney were generally low (0.00-0.08 ppm), while the concentrations of radioactivity in fat were somewhat higher (average for all treatments ~0.47 ppm). Male and female rats treated with 0.1 mg ^{14}C-HCS-3260/kg bw had higher tissue residue levels of radioactive equivalents in liver (0.50 ppm), kidney (0.26 ppm) and fat (3.71 ppm for females, 2.58 ppm for males). In general, female rats accumulated greater concentrations of radioactivity in fat than male rats after treatment with any preparation. At 7 days after dosing with 1.0 mg ^{14}C-HCS-3260/kg bw, radioactivity in all tissues declined; radioactivity in fat declined to 2.0 ppm for females and 1.19 ppm for males. Slightly more radioactivity was present in rat tissues following oral doses of trans- than cis-chlordane-^{14}C.

When ^{14}C-HCS-3260 was administered to male rats in the diet at 5 ppm (5 mg/kg diet) for 56 days, the tissue distribution of radioactive equivalents was 0.42, 0.91, 0.55, 0.68 and 14.73 ppm for muscle, brain, kidney, liver and fat, respectively (Barnett and Dorough, 1974). After discontinuing treatment for 28 and 56 days, the concentration of radioactivity in fat declined to 3.67 and 2.49 ppm, respectively. Radioactivity was still detected in other tissues 56 days after treatment was terminated. Greater accumulation of radioactivity in all tissues resulting from trans- rather than from cis-chlordane-^{14}C occurred in female rats treated with 25 ppm (25 mg/kg diet). Analysis of the nature of the radioactivity revealed that ~30-60% of the radiocarbon was associated with oxychlordane.

Ambrose et al. (1953b) found that the perirenal fat of male rats contained 43, 41 and 81 ppm of chlordane residues following feeding for 5, 148 and 407 days, respectively, of a diet containing 320 ppm (320 mg/kg diet). The fat of female rats contained approximately twice the values for males.

Residues of parent isomers and oxychlordane in adipose tissue from male and female Holtzmann rats maintained on diets containing 50-200 ppm (50-200 mg/kg diet) cis- or trans-chlordane, or 100 ppm (100 mg/kg diet) of fixed ratios of the isomers from 9:1 to 1:9 trans:cis ratios, or 50 ppm (50 mg/kg diet) technical chlordane for 15 days were determined by Street and Blau (1972). Adipose tissue of female rats fed trans-chlordane at 50, 100 and 200 mg/kg diet contained ~6, 10 and 23 μg/g lipid of trans-chlordane, respectively. Much greater concentrations of oxychlordane than trans-chlordane were stored in adipose tissue by females (~104, 202 and 471 μg/g lipid) and males (~5, 15 and 22 μg/g lipid) after being fed trans-chlordane. The feeding of cis-chlordane likewise resulted in greater fat storage of the metabolite than of the parent isomer in both male and female rats; however, the ratio oxychlordane:isomer was reduced. Female rats fed 50 ppm technical grade chlordane (50 mg/kg diet) stored ~7 times more oxychlordane than the parent compound in adipose tissue. The results of feeding ratios of cis- and trans-chlordane indicated that oxychlordane accumulation was additive for each isomer.

Polen et al. (1971) also detected oxychlordane in the fat depots of rats, dogs and pigs maintained on diets containing individual isomers or technical chlordane. Rats received dietary concentrations of cis-, trans- or technical grade chlordane of 0-150 ppm (0-150 mg/kg diet) for 1 year; dogs received technical grade chlordane at 3 or 30 ppm (3 or 30 mg/kg diet) for 2 years; pigs were fed diets containing the cis- or trans-isomer at 300 ppm (300 mg/kg diet) for 90 days. The respective levels of oxychlordane in the fat for rats, dogs and pigs were 0.2-150, 3-24 and 36-90 ppm.

Rabbits received trans-chlordane-^{14}C daily per os in doses of 14.3 mg/rabbit/day for 10 weeks (Poonawalla and Korte, 1971). Two weeks after treatment was discontinued, low levels of radioactivity were detected in kidney (0.05%), liver (0.52%), heart (0.09%), lung (0.04%), spleen (0.03%), testes (0.03%) and brain (0.04%). Higher levels were found in adipose tissue (2.59% in abdominal fat, 1.53% in subcutaneous fat) and muscle (5.68%). Barnett and Dorough (1974) found that the tissues of a rabbit fed ^{14}C-HCS-3260 (25 mg/kg diet for 2 days) contained ^{14}C-oxychlordane and ^{14}C-dichlorochlordene.

The distribution of radioactivity in the tissues of rabbits administered cis- or trans-chlordane-^{14}C orally in four doses, one given every 4 days, of 100 mg in a capsule per rabbit was as follows: fat > kidney > muscle > liver > brain (cis-); kidney > fat > liver > muscle > brain (trans-) (Balba and Saha, 1978). The majority of the radioactivity in the tissues was associated with oxychlordane, regardless of which isomer was administered.

The mobilization of chlordane or its metabolites from adipose tissue of rats after food deprivation was studied by Ingle (1952). Chlordane was removed from the diet after 80 weeks maintenance of 16 rats on a diet containing chlordane at 150 ppm (150 mg/kg diet). At weekly intervals, two male and two female rats were fasted for 48 hours and observed for symptoms of toxicity. After 4 weeks, symptoms such as tremors and hyperactivity were no longer observed.

The distribution of radioactivity (expressed as % of administered radioactivity/tissue) in selected tissues of rats following the previously described intratracheal administration of ^{14}C-chlordane (Nye and Dorough, 1976) was 23.9% in lungs, 19.6% in liver, 0.3% in kidney and 0.1% in the bladder and its contents.

Metabolism -- The metabolism of chlordane has been fairly well studied in rats and rabbits in vivo and in vitro. Oxychlordane was a metabolite of both cis- and trans-chlordane (Street and Blau, 1972). A metabolic pathway, based upon in vitro studies with rat liver homogenates, was proposed in which each isomer is dehydrogenated to 1,2-dichlorochlordene with subsequent epoxidation to oxychlordane. In these experiments, trans-chlordane was converted to oxychlordane at a 7-fold greater rate than was the cis-isomer.

Barnett and Dorough (1974) isolated seven radioactive metabolites (in addition to the respective unchanged parent compounds) in the feces of rats that had been administered cis- or trans-chlordane-^{14}C or the 3:1 mixture of cis- and trans-chlordane, designated as ^{14}C-HCS-3260, either as single oral doses (0.2 mg/kg bw) or by continuous feeding (5 mg/kg diet of ^{14}C-HCS-3260 for 56 days or 25 mg/kg diet cis- or trans-chlordane-^{14}C for 14 days). These metabolites, analyzed by TLC and GLC, were tentatively identified as hydroxychlordane; chlordene chlorohydrin; monochloro and dihydroxy derivatives of chlordane; cis- and/or trans-dihydroxychlordane derivatives; a trihydroxylated chlordene; and a conjugated form of a hydroxylated chlordane metabolite. No oxychlordane or dichlorochlordene was detected in feces; however, oral administration of oxychlordane resulted in fecal excretion of unchanged oxychlordane. The nature of urinary metabolites was essentially the same as fecal metabolites in rats fed HCS-3260 in dietary concentrations of 25 mg/kg diet: oxychlordane was also present. The 24-hour feces of a rabbit fed ^{14}C-HCS-3260 at 25 mg/kg diet for 2 days contained the same fecal metabolites found in the rat, although the amounts of unchanged isomers were greater. The urine of the rabbit contained a greater percentage of the conjugated hydroxylated metabolites than did the urine of rats.

In the previously described study in rabbits, Balba and Saha (1978) identified the following urinary metabolites of cis-chlordane: 1-hydroxy-2-chlorochlordene, trans-chlordene chlorohydrin and 1-hydroxychlordene. Urinary metabolites in rabbits of trans-chlordane were 1-hydroxy-2-chlorochlordene, 1,2-dichlorochlordene, trans-chlordene chlorohydrin and 3-hydroxychlordane.

The metabolism of cis- and trans-chlordane-^{14}C was also studied in rats _in vivo_ and _in vitro_ by Tashiro and Matsumura (1977). Male rats maintained on diets containing cis- or trans-chlordane-^{14}C in a concentration of 100 ppm (100 mg/kg diet) for 4 weeks excreted 13 metabolites from cis- and 14 metabolites from trans-chlordane in the feces. The fecal metabolites of both isomers, identified by TLC and GLC, included heptachlor, 1,2-dichlorochlordene, oxychlordane, 1-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, chlordene chlorohydrin, monohydroxy dihydrochlordene, 1,2-dihydroxychlordene and trihydroxydihydrochlordene. These metabolites were found in different proportions, depending upon the administered isomer. The only urinary metabolite identified from both cis- and trans-chlordane was a glucuronide conjugate of 1-hydroxydihydrochlordene. The _in vitro_ incubation of the isomers with rat liver microsomes and cofactors resulted in the same metabolites qualitatively.

Based upon these _in vivo_ and _in vitro_ results, Tashiro and Matsumura (1977) concluded that chlordane is dehydrogenated to dichlorochlordene, which is epoxidized at carbons 2 and 3 and hydroxylated at carbon 1 (Route A). An additional route whereby chlordane is hydroxylated at carbon 1 to yield 1-hydroxydihydrochlordene was also proposed (Route B). These pathways are illustrated in Figure III-1.

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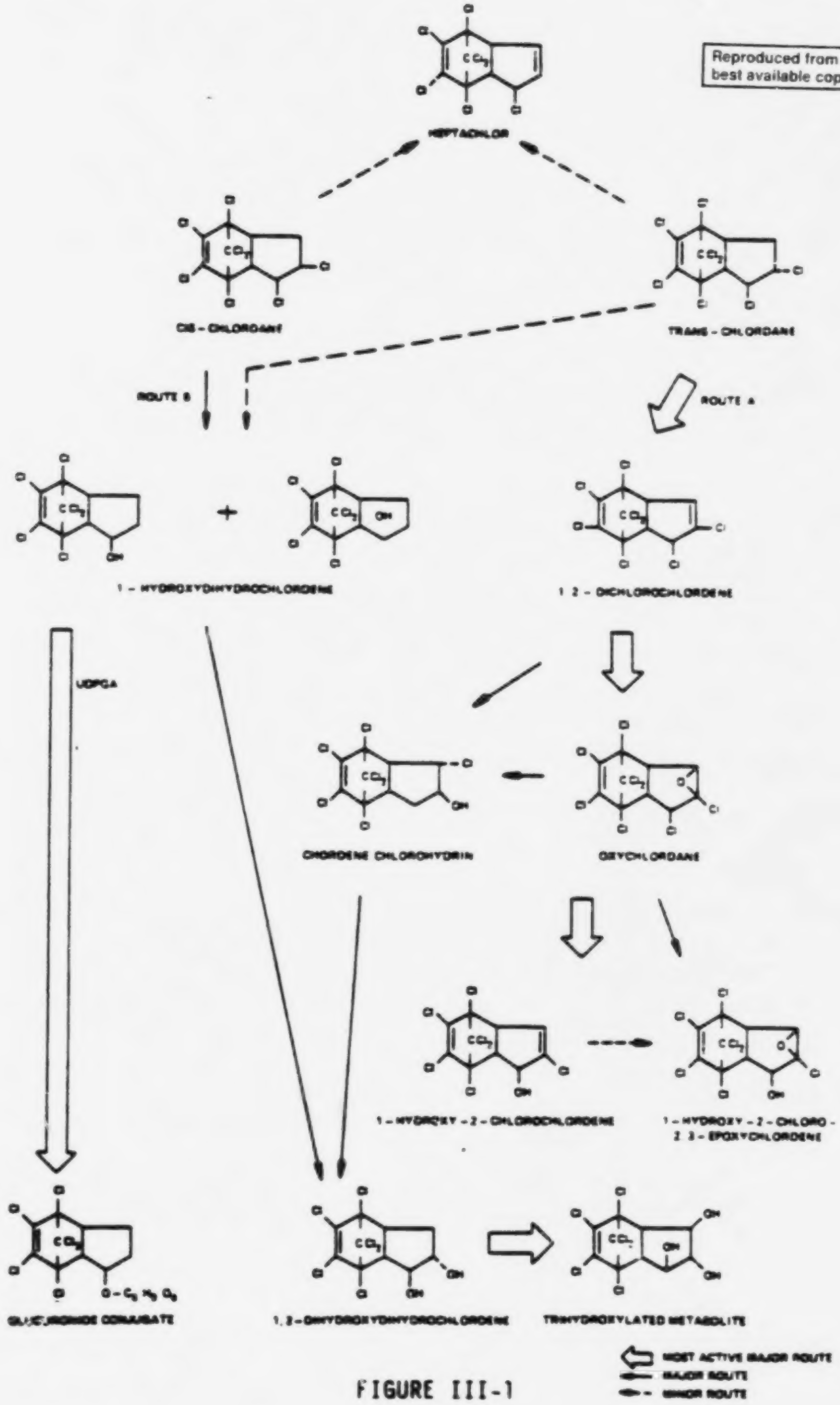

HEPTACHLOR
CIS - CHLORDANE
TRANS - CHLORDANE
ROUTE B
ROUTE A
1 - HYDROXYDIHYDROCHLORDENE
1, 2 - DICHLOROCHLORDENE
UDPGA
CHORDENE CHLOROHYDRIN
OXYCHLORDANE
1 - HYDROXY - 2 - CHLOROCHLORDENE
1 - HYDROXY - 2 - CHLORO -
2, 3 - EPOXYCHLORDENE
GLUCURONIDE CONJUGATE
1, 2 - DIHYDROXYDIHYDROCHLORDENE
TRIHYDROXYLATED METABOLITE
MOST ACTIVE MAJOR ROUTE
MAJOR ROUTE
MINOR ROUTE

FIGURE III-1

Proposed Metabolic Pathways for Chlordane

Source: Tashiro and Matsumura, 1977

Brimfield et al. (1978) also studied the in vitro metabolism of the pure cis- and trans-isomers of chlordane by microsomal preparations from Sprague-Dawley rats fed the individual isomers in dietary concentrations of 100 ppm (100 mg/kg diet) for 9 days. Incubation of cis-chlordane-induced enzymes with cis-chlordane resulted in the following metabolites, identified by GC-MS: cis-chlordane, dichlorochlordene, oxychlordane, dihydroheptachlor, hydrochlordene and 1-chloro-2-hydroxydihydrochlordene. Similar products from trans-chlordene were identified; however, heptachlor rather than dihydroheptachlor was formed, as was hydroxychlordane. A metabolic pathway, based upon reductive dechlorination via dihydroheptachlor to dihydrochlordene, was proposed. According to this scheme, these molecules can also be hydrolyzed, desaturated and epoxidized. In the Brimfield et al. (1978) proposal, oxychlordane is an endpoint, whereas, in the scheme proposed by Tashiro and Matsumura (1977), oxychlordane undergoes further hydroxylation and reduction. Many of the proposed intermediates in the Brimfield scheme have not actually been found in this or other studies (Brimfield et al., 1978).

Excretion -- In the previously described experiments of Barnett and Dorough (1974), >90% of the administered radioactivity was excreted over 7 days by rats that were given single oral doses (0.2 mg/kg diet) of cis- or trans-chlordane-^{14}C or ^{14}C-HCS-3260. Females excreted 5-6% of the radioactivity in the urine; males excreted 2-3%. At higher doses (0.5 or 1.0 mg/kg diet) of HCS-3260, the pattern of elimination was essentially the same. When ^{14}C-HCS-3260 was fed in the diet for 56 days, fecal elimination as measured by radioactivity was 70% for the 1 mg/kg diet level, 75%

for the 5 mg/kg diet level and 80% for the 25 mg/kg diet level, indicating possible decreased absorption: the investigators made no mention of possible biliary excretion. Elimination of cis-chlordane (75%) was greater than trans-chlordane (65%) following 14 days of dietary levels of the isomers at 25 mg/kg diet.

Tashiro and Matsumura (1977) reported similar results for elimination from rats treated with single oral doses of cis- (5.4 mg/kg bw) and trans-chlordane-^{14}C (9.7 mg/kg bw) in corn oil. The total 7-day elimination of radioactivity from cis- and trans-chlordane was 86 and 66% of the administered dose, respectively. The 24-hour total excretion was 59% for cis- and 27% for trans-chlordane.

Rabbits excreted 18% of an orally administered single dose of 200 mg/kg bw of chlordane in the urine collected over 16 days when organic chlorine content was measured (Stohlman et al., 1950). The peak urinary elimination of organic chlorine occurred within 2 days and amounted to 9% of the dose.

Poonawalla and Korte (1971) observed appreciable urinary excretion of radioactivity by rabbits that received 14.3 mg/rabbit of trans-chlordane-^{14}C daily for 10 weeks. At the end of this period, ~70% of the daily dose had been eliminated: 22.7% was excreted in the feces, 30% of which was unchanged trans-chlordane; and 47% was excreted as urinary metabolites. In agreement with these results, excretion of radioactivity by one rabbit 24 hours after termination of feeding 25 ppm (25 mg/kg diet) of HCS-3260-^{14}C for 2 days was 21% in feces and 33% in urine (Barnett and Dorough, 1974).

Balba and Saha (1978) also observed appreciable urinary excretion of the ^{14}C-label from cis- or trans-chlordane-^{14}C by rabbits that were treated with either isomer orally at a dose of 100 mg/rabbit in a capsule every 4 days up to 400 mg/rabbit; however, in this study, urinary excretion did not exceed fecal excretion. For the cis-isomer, 48.5% and 28.4% of the radioactivity were eliminated in the feces and urine, respectively. For the trans-isomer, fecal and urinary excretion were 46.1% and 35.8%, respectively.

When an unspecified amount of chlordane-^{14}C in 20 µℓ of ethanol was administered intratracheally to female Sprague-Dawley rats, elimination of radioactivity was primarily in the feces (Nye and Dorough, 1976). After a lag period of 2 days during which <20% of the dose was eliminated, fecal excretion rose to ~50% by day 4 and 56% by day 6. Urinary excretion over 6 days amounted to 12% of the dose. No radioactivity was detected in the expired air of these animals.

Heptachlor and Heptachlor Epoxide.

Absorption -- Limited information on the absorption of heptachlor following ingestion by animals was available. A Russian study (Mizyukova and Kurchatov, 1970) has reported that heptachlor administered intragastrically in a single oral dose of 120 mg/kg bw to rats was detected in blood within 0.5-1 hour of administration.

The absorption of heptachlor following inhalation exposure also has not been well studied. From July 1, 1972 to October 4, 1972, Arthur et al. (1975) placed 10 rabbits of each sex in open-air cages so that they were exposed to the ambient air of Stoneville, Mississippi, an area where insecticides had been heavily used. Control groups of male and female rabbits (10 each) were housed in a room at Mississippi State University, a low pesticide use area. The average air levels of heptachlor epoxide (heptachlor was either not measured or not detected) in Stoneville air was 1.86 ng/m³; the air at Mississippi State was not sampled. Heptachlor epoxide residue levels in adipose tissue of the test rabbits was 0.039 ppm compared with 0.016 ppm in controls ($p<0.001$). The average respiratory intake of heptachlor epoxide was calculated as 0.002 µg/day for rabbits in the Stoneville area.

Distribution and Retention -- An abstract of a Russian study (Mizyukova and Kurchatov, 1970), reported that following a single intragastric dose of 120 mg/rat heptachlor to female rats, heptachlor was detected in blood, liver, kidney and adipose tissue within 1 hour. After 4 hours, heptachlor epoxide, a metabolite of heptachlor, was detected in blood, liver and fat and persisted in the adipose tissue for 3-6 months.

Radomski and Davidow (1953) studied the tissue distribution of heptachlor epoxide in rats and dogs following oral administration of heptachlor. Nine rats of either sex received dietary concentrations of heptachlor at 30-35 ppm (30-35 mg/kg diet) for 2 months. Heptachlor epoxide levels in the fat of six female rats averaged 384 µg/g tissue, while fat in male rats contained an average of only 43 µg/g tissue. Much lower levels of heptachlor epoxide were detected in liver (0.4-33 µg/g), kidney (0-21 µg/g) and muscle (0-27 µg/g). None was detected in brain tissue. A similar pattern of distribution was observed in three female dogs that received 1 mg heptachlor/kg bw in capsules daily for 12-18 months. Fat contained an average heptachlor epoxide concentration of 636 µg/g; liver, 36 µg/g; kidney, 7.6 µg/g; and muscle, 13 µg/g. Again, no heptachlor epoxide was detected in the brain. Small amounts of unchanged heptachlor were detected in the fat of dogs when higher levels of the insecticide were administered. Administration of 1 mg heptachlor/kg bw to four dogs resulted in no detectable heptachlor in fat after 26 weeks, while three of eight dogs, surviving 6-18 weeks, dosed with 3 mg/kg bw daily had <1 µg heptachlor/g fat tissue. A daily dose of 5 mg/kg bw resulted in an average concentration of heptachlor in fat of 6.5 µg/g. This dose was fatal to the dogs within 2-11 weeks.

Radomski and Davidow (1953) also determined the rate of accumulation and disappearance of heptachlor epoxide in rats (three rats/sex/group) fed a diet containing 30 ppm (30 mg/kg diet) heptachlor. One group was sacrificed each week for 12 weeks. Female rats accumulated heptachlor epoxide to a maximum level of ~225 µg/g fat in 8 weeks compared with only 50 µg/g fat in males. When the test diet was discontinued, heptachlor epoxide was no longer detected at 8 weeks for female fat tissue and 6 weeks for male fat tissue. Further tests established that the maximum dietary level at which fat storage did not occur was 0.3 mg/kg diet for male rats and 0.1 mg/kg diet for female rats.

Although Radomski and Davidow (1953) failed to detect heptachlor epoxide in rat and dog brain tissue after heptachlor treatment, Yamaguchi et al. (1980), using a GC method, detected 3.15 ppm heptachlor epoxide in brain tissue of rats 5 hours after an intraperitoneal injection of 200 mg heptachlor/kg bw.

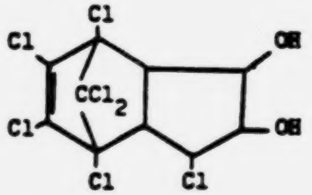
Metabolism -- Davidow and Radomski (1953) first identified heptachlor epoxide in the adipose tissue of dogs that had been treated with daily oral doses of 1-3 mg heptachlor/kg bw in corn oil. They concluded that the metabolite arose by the epoxidization of heptachlor. Subsequently, these investigators (Radomski and Davidow, 1953) also isolated heptachlor epoxide from the adipose tissue of male and female rats maintained on diets containing 30-35 ppm (30-35 mg/kg diet) heptachlor.

In an abstract of a Russian study, Ermakov (1977) reported that heptachlor, administered to rats and rabbits in single oral doses of 28-50 mg/kg bw, was metabolized by reactions involving hydrolysis, hydroxylation, epoxidation, dehydrogenation and molecular rearrangement, but details as to intermediate structures were not given in the abstract.

The most extensive investigation of the metabolic fate of heptachlor in rats was performed by Tashiro and Matsumura (1978) in vivo and in vitro. Over a 10-day period following a single oral unspecified dose of ^{14}C-heptachlor (position of label not specified) in corn oil, rats excreted >50% of the administered radioactivity in feces, and <5% in urine. The relative abundance of fecal metabolites, expressed as percent of ^{14}C-compounds, were as follows: unchanged heptachlor, 26.2%; heptachlor epoxide, 13.1%; 1-hydroxychlordene, 19.5%; 1-hydroxy-2,3-epoxychlordene, 17.5%; 1,2-dihydroxydihydrochlordene, 3.5%; and two other unnamed metabolites, one of which accounted for 19% of the radioactivity, the other for <0.1%. The latter metabolite was designated as H-2 by these authors, and its structure is identical to that proposed by Matsumura and Nelson (1971) for what they called the fecal metabolite of heptachlor epoxide. The *in vitro* metabolism of ^{14}C-heptachlor by rat liver microsomal preparations resulted in the following relative abundance of metabolites, expressed as % of the total ^{14}C-compounds: heptachlor, 4.4%; heptachlor epoxide, 85.8%; 1-hydroxychlordene, 2.9%; 1-hydroxyepoxychlordene, 3.0%; 1,2-dihydroxydihydrochlordene, 0.9%; and an unknown metabolite, 3.0% (Tashiro and Matsumura, 1978). No H-2 fecal metabolite was detected. The identity of the metabolites was confirmed by TLC analysis and comparison with authentic standards of fecal metabolites from rats fed diets containing 100 ppm (100 mg/kg diet) heptachlor for 4 weeks. A pathway of heptachlor metabolism by rats was proposed and is illustrated in Figure III-2.

Matsumura and Nelson (1971) administered heptachlor epoxide to four rats in dietary concentrations of 10 ppm (10 mg/kg diet) for 30 days. The authors estimated that each rat consumed 5 mg of heptachlor epoxide over the test period and excreted 950 μg of a fecal metabolite (see Figure III-2) and 66 μg of heptachlor epoxide in the feces.

Brooks et al. (1968, 1970) investigated the *in vitro* metabolism of heptachlor epoxide by pig liver microsomes. The product, formed upon incubation at 45°C for 60 hours, was identified as heptachlor epoxide diol:



Incubation of heptachlor epoxide with rabbit microsomes also resulted in the formation of heptachlor epoxide diol as well as another unidentified product.

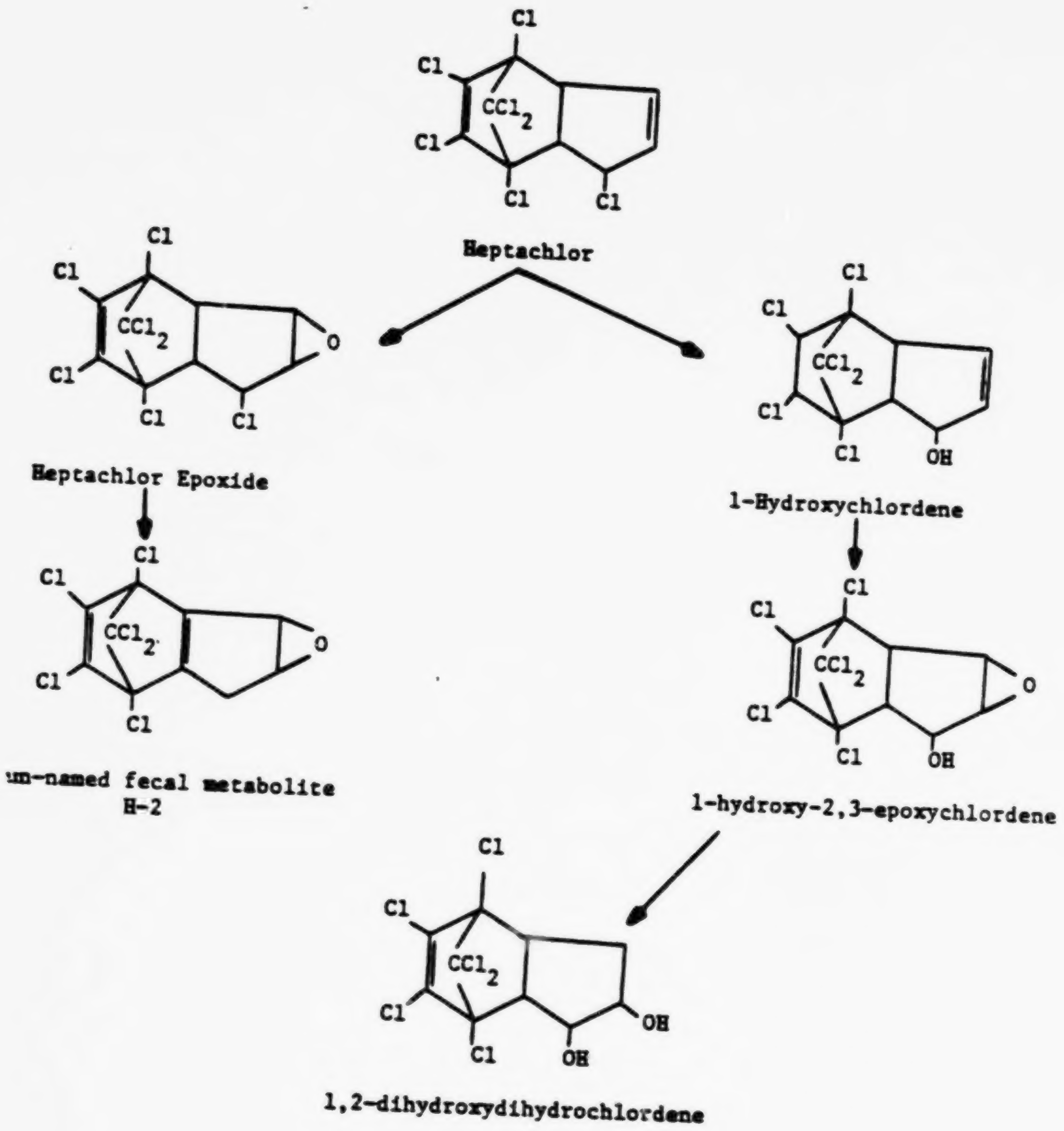

Heptachlor
Heptachlor Epoxide
1-Hydroxychlordene
un-named fecal metabolite
H-2
1-hydroxy-2,3-epoxychlordene
1,2-dihydroxydihydrochlordene
Cl
CCl_2
OH
O

FIGURE III-2

Proposed Metabolic Pathway of Heptachlor in Rats

Source: Tashiro and Matsumura, 1978

Excretion -- Tashiro and Matsumura (1978) reported that rats excreted >50% of the administered single oral unspecified dose of radioactivity from ^{14}C-heptachlor in the feces over 10 days. Urinary excretion of radioactivity accounted for <5% of the administered amount.

In an abstract of a Russian study (Ermakov, 1977), it was reported that 16-40% of the orally administered dose of heptachlor (28-50 mg/kg bw) to rats and rabbits was excreted unchanged in the feces and that heptachlor epoxide was excreted over at least a 12-month period. Further details were not given in the abstract. As reported in an abstract of another Russian study, Mizyukova and Kurchatov (1970) showed that heptachlor and its metabolites were excreted mainly in the feces of rats within 5 days of an intragastric dose of 120 mg/kg bw.

Each of four male albino rats excreted an average of 950 μg of the fecal metabolite (see Figure III-2) of 99% pure heptachlor epoxide during 30 days of maintenance on a diet containing 10 ppm (10 mg/kg diet) heptachlor epoxide (each rat consumed ~5 mg over 30 days) (Matsumura and Nelson, 1971). Approximately 66 μg of unchanged heptachlor epoxide was also excreted in the feces.

Human Data

Chlordane.

Absorption -- A 20-month-old boy (12.7 kg) drank an unknown quantity of 74% technical grade chlordane from a bottle (Curley and Garrettson, 1969). He vomited 45 minutes later and convulsions began shortly thereafter. He was admitted to the hospital 75 minutes after ingestion of the pesticide. The concentration of chlordane in a blood sample taken 2.75 hours after ingestion was 2.71 mg/ℓ. The concentration in fat was 3.12 mg/kg bw. A 4-year-old girl (11 kg) drank an unknown amount of a 45% chlordane formulation (Aldrich and Holmes, 1969). She was admitted to the hospital 3 hours later, and gastric lavage was performed. The first serum sample taken (time not specified) contained 3.4 ppm (mg/kg serum), from which the investigators calculated an absorbed quantity of at least 1.62 mg.

Distribution and Retention -- Information on the distribution of chlordane to tissues following chlordane ingestion is available from a case report of accidental poisoning and from autopsy samples.

In the previously described case of the 20-month-old boy who drank an unknown quantity of 74% chlordane, Curley and Garrettson (1969), assuming that fat comprised 15% of the body weight, calculated that 5.9 mg chlordane was in the fat 3 hours after ingestion; 36 mg chlordane was present on the next day, and 65.0 mg chlordane was in fat 8 days after ingestion. Three months after ingestion, the concentration of chlordane in fat was 25.53 mg/kg. At this time the ratio of chlordane in fat to serum was 1470:1. No fat samples were collected by Aldrich and Holmes (1969) from the 4-year-old girl who ingested chlordane.

Biros and Enos (1973) reported that of 27 adipose tissue specimens obtained at autopsies from people of the general population, 21 were positive for oxychlordane, a metabolite of chlordane, with a mean concentration of 0.14±0.09 ppm and a range of 0.03-0.40 ppm. The mean adipose tissue levels of oxychlordane in 10 autopsy samples from the Dade County, Florida, white female population in 1978 was 0.193 ppm, with a range of 0.07-0.49 ppm (Barquet et al., 1981). Cis- and trans-chlordane levels were below the limits of GC-electron capture detection (<0.10 ppm). Sovocool and Lewis (1975) also detected oxychlordane in human adipose tissue obtained at autopsy from several hospitals.

__Metabolism__ -- The _in vitro_ metabolism of cis- and trans-chlordane-^{14}C by rat and human liver microsomal preparations was compared by Tashiro and Matsumura (1978). Their data are presented in Table III-1. Examination of the table reveals very little interspecies difference between the rat and human _in vitro_ metabolism of the two isomers.

Excretion -- In the case of accidental poisoning of a 4-year-old, 11 kg girl by ingestion of an unknown quantity of a 45% chlordane formulation (Aldrich and Holmes, 1969), a nonlinear decline of serum chlordane concentration from 3.4 to 0.138 mg/kg serum in the first 3 days and 0.03 mg/kg serum by 130 days was observed. During the last 90 days of this period, the concentration of chlordane in serum declined at a rate of 0.036 mg/kg day, and a biological half-life was estimated as 88 days. The chlordane concentration in urine declined from 1.93 to 0.05 ppm over the first 3 days after ingestion; however, 35 days later the urinary level was 0.13 ppm. The investigators speculated that the rise may have resulted from release of stored chlordane. On days 2 and 3 after ingestion, fecal chlordane levels were 719 and 105 ppm, respectively. No chlordane was detected in the feces 1 or 2 months later.

TABLE III-1

Metabolism of cis-Chlordane and trans-Chlordane *in vitro* by Human and Rat Liver Microsomal Preparations: The Data Are Expressed as Percent of the Total Starting Material[a,b]

Starting Material and Metabolites	cis-Chlordane		trans-Chlordane	
	Human[c]	Rat	Human[d]	Rat
1,2-Cl-chlordene	0.5	2.1	0.4	NA
Oxychlordane	1.0	5.2	0.4	2.3
cis-Chlordane	83.1	85.5	NA	NA
trans-Chlordane	NA	NA	85.25	86.4
C-4 (T-4)[e]	0.5	0.6	1.0	0.8
C-5 (T-5)[f]	0.1	0.1	0.2	0.1
HM[g]	0.6	NA	2.5	NA
Chlordene chlorohydrin	5.7	3.8	4.5	7.9
C-10 (T-10)[h]	2.9	0.9	0.8	1.3
1,2-OH-chlordene	0.4	0.3	0.4	0.3
C-12 (T-12)[i]	0.6	0.2	0.5	0.0
C-13 (T-13)[j]	4.6	1.3	3.5	0.8

[a] Source: Tashiro and Matsumura, 1978

[b] Microsomal fraction prepared by centrifuging human liver tissue homogenate at 20,000 x g for 20 minutes. Incubation mixture made up to 5 ml with incubation at 37°C in shaking incubator for 2 hours.

[c] Patient A only

[d] Average of two patients (A and B). The amounts of trans-chlordane remaining were 86.6% for A and 83.9% for B.

[e] C-4, T-4 = 1-hydroxy-2-chlorochlordene derived from cis- or trans-chlordane

[f] C-5, T-5 = 1-hydroxy-2-chloro-2,3-epoxychlordene

[g] Unknown metabolite found only in human experiments

[h] C-10, T-10 = monohydroxylated dihydrochlordene

[i] C-12, T-12 = trihydroxydihydrochlordene

[j] C-13, T-13 = beta-glucuronide-1-hydroxydihydrochlordene

NA = Not applicable

In the case of the 20-month-old boy who ingested an unknown quantity of 74% chlordane, Curley and Garrettson (1969) estimated the half-life of chlordane in serum to be 21 days. The concentration of chlordane in the 24-hour urine was 0.309 mg/ℓ, corresponding to 48.8 μg chlordane. Further urine samples were not collected.

In a survey conducted in Arkansas and Mississippi in 1973 and 1974, Strassman and Kutz (1977) reported that of 57 human milk samples, 45.6% were positive (quantifiable) for oxychlordane residues, while 54.4% contained unquantifiable traces. The mean level of oxychlordane residues in positive samples was 0.012 ppm. Thus, a route of chlordane or oxychlordane excretion in human females is lactation. The investigators stated that, to their knowledge, this was the first report of oxychlordane occurrence in human milk.

Heptachlor and Hepachlor Epoxide.

Absorption -- No information on the absorption of heptachlor or heptachlor epoxide by humans was available. That these insecticides are absorbed can be inferred from the many reports of adipose tissue levels of heptachlor epoxide, although the route cannot be readily ascertained.

Distribution -- Klemmer et al. (1977) reported that heptachlor epoxide was detected in tissue samples from 77 autopsies performed from 1966-1968 in a range of concentrations of 1-32 ppb whole tissue. The highest concentrations occurred in bone marrow and liver.

Heptachlor epoxide has been detected in human adipose tissue in a large number of surveys conducted in Great Britain (Abbott et al., 1972; 1981), Brazil (Wassermann et al., 1972), Japan (Curley et al., 1973), Israel (Wassermann et al., 1974), Texas (Burns, 1974), Louisiana (Greer et al., 1980) and the continental United States (Kutz et al., 1979, Sovocool and Lewis, 1975).

Several studies have reported transplacental transfer of heptachlor or heptachlor epoxide in humans. Curley et al. (1969) detected heptachlor epoxide in adipose tissue, brain, adrenals, lungs, heart, liver, kidney and spleen of 10 stillborn babies and 2 babies who died soon after birth and in 27 of 30 samples of cord blood from healthy neonates. All concentrations fell within the range of levels detected in the general adult population. Others have also reported that heptachlor epoxide levels in tissues of stillborn and soon dead infants correspond to concentrations found in adults (Zavon et al., 1969; Wassermann et al., 1972, 1974). A comparison of heptachlor and heptachlor epoxide residues in 53 maternal blood and 54 placental tissue samples revealed that, in general, high levels of the compounds in maternal blood were reflected as high levels in placentae and vice versa (Selby et al., 1969). The ratio of the concentration of heptachlor epoxide in placentae to the concentration in maternal blood was 5.8:1, based upon geometric means. For total equivalents of heptachlor, defined as the sum of the values for heptachlor and heptachlor epoxide, the ratio was 8.8:1. Polishuk et al. (1977a) reported mean levels of heptachlor epoxide in the extracted lipid of tissues from 24 women during labor as follows: adipose tissue, 0.2856 ppm; maternal blood, 0.2798 ppm; fetal blood, 0.9959 ppm; uterine muscle, 0.4895 ppm; placenta, 0.5000 ppm; and amniotic fluid 0.6730 ppm.

Metabolism -- The results of a comparison of the *in vitro* metabolism of heptachlor by human and rat liver microsomal preparations (Tashiro and Matsumura, 1978) are presented in Table III-2. A major difference between the species is that 4 times more heptachlor epoxide was formed in the rat system than in the human system. The investigators pointed out that since heptachlor epoxide is a stable product not involved in the major metabolic pathway of heptachlor (see Figure III-2), the relative ease of its formation may influence the relative degree to which heptachlor and heptachlor epoxide are stored by adipose tissue.

Excretion -- Information regarding the urinary and fecal excretion of heptachlor and heptachlor epoxide by humans was not available. Excretion of heptachlor epoxide in the milk of lactating mothers, however, has been reported by several investigators. Kroger (1972) detected an average of 0.16 ppm heptachlor epoxide in 53 human milk samples. A mean concentration of 0.003 mg/kg of heptachlor epoxide in milk was reported by Ritcey et al. (1972) in 147 human milk samples collected in Canada in 1967-1968. Heptachlor epoxide, in trace amounts up to concentrations of 5 ppb, was detected in 10 of 40 milk samples collected from women in Colorado in 1971-1972 (Savage et al., 1973). The mean concentration of heptachlor epoxide in 18 of 50 human milk samples collected in Norway was 1.57 ppb (Bakken and Seip, 1976). In Israel in 1975, a mean concentration of 0.0091 ppm was detected in whole milk collected from 29 women 2-4 days after delivery (Polishuk et al., 1977b). Of 51 human milk samples collected in St. Louis, MO, in 1973, 12 were positive for heptachlor epoxide (mean concentration = 0.0027 ppm) and 3 were positive for heptachlor (mean concentration = 0.019 ppm) (Jonsson et al., 1977). Strassman and Kutz (1977) detected heptachlor epoxide in

TABLE III-2

Heptachlor Metabolism *in vitro* by Human and Rat Liver Microsomal Preparations: The Data Are Expressed as Percent of Starting Material*

Starting Material and Metabolites	Human	Rat
Heptachlor	68.6	4.4
H-1 (heptachlor epoxide)	20.4	85.8
H-2	<0.1	--
H-3 (1-OH-chlordene)	4.8	2.9
H-4 (1-OH-chlordene epoxide)	5.0	3.0
H-5 (1,2-OH-chlordene)	0.1	0.9
H-6	1.0	3.0

*Source: Tashiro and Matsumura, 1978

35.1% of 57 human milk samples from Arkansas and Mississippi in 1973-1974 in a mean concentration of 0.004 ppm. All milk samples from 50 lactating women in Hawaii collected from 1979-1980 contained heptachlor epoxide in a mean concentration of ~34 ppb (Takahashi et al., 1981). Lactation may, therefore, represent an important route of excretion of heptachlor epoxide in nursing mothers and reduce the body burden in this human subpopulation. On the other hand, breast feeding results in an additional source of pesticide exposure for infants.

Summary

Chlordane. The absorption of chlordane by the gastrointestinal tract of animals can be inferred from data on its systemic toxicity and from excretion data following ingestion. At least 2-8% of orally administered ^{14}C-chlordane was absorbed by rats and at least 30% was absorbed by rabbits, as evidenced by the percent radioactivity recovered in urine (Barnett and Dorough, 1974). Chlordane was absorbed by the pulmonary route by rats (Nye and Dorough, 1976). More chlordane in lipophilic vehicle than in ethanol was absorbed by rats dermally (Ambrose et al., 1953b). Information on the absorption of chlordane by humans following ingestion is available from case studies of accidental poisoning of children (Curley and Garrettson, 1969; Aldrich and Holmes, 1969).

Once absorbed, chlordane and/or its metabolites are rapidly distributed throughout the body with highest levels detected in adipose tissue (Barnett and Dorough, 1974). Radioactivity in all tissues declined when treatment was discontinued, but detectable levels were still present in all tissues of rats 56 days after termination of feeding ^{14}C-chlordane for 56 days.

Female rats accumulated greater concentrations of chlordane or metabolites than did male rats (Barnett and Dorough, 1974; Ambrose et al., 1953b; Street and Blau, 1972). Greater accumulation of residues by rats from trans- than from cis-chlordane was observed (Barnett and Dorough, 1974; Street and Blau, 1972). The major form of chlordane stored in tissues has been identified as oxychlordane (Barnett and Dorough, 1974; Street and Blau, 1972; Polen et al., 1971). Similar results have been observed in dogs, pigs (Polen et al., 1971) and rabbits (Poonawalla and Korte, 1971; Barnett and Dorough, 1974) and in rats following pulmonary absorption (Nye and Dorough, 1976). Oxychlordane has been detected in adipose tissue of the general human population obtained at autopsy (Biros and Enos, 1973; Barquet et al., 1981; Sovocool and Lewis, 1975). Chlordane was stored for at least 3 months in the fat of one child who drank a formulation of the insecticide (Curley and Garrettson, 1969).

Oxychlordane is a metabolite of both cis- and trans-chlordane in rats *in vivo* and *in vitro*, although more oxychlordane is formed from trans-chlordane (Street and Blau, 1972; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977; Brimfield et al., 1978). At least 13 or 14 fecal metabolites have been isolated, many of which have been identified; they are identical, whether formed from cis- or trans-chlordane. A glucuronide conjugate was found in the urine (Tashiro and Matsumura, 1977). A metabolic scheme involving dehydrogenation, epoxidation, hydroxylation and dechlorination reactions was presented. Metabolism of chlordane *in vitro* by human liver microsomal preparations was found to be very similar to rat microsomal *in vitro* metabolism (Tashiro and Matsumura, 1978).

The major route of elimination of chlordane and its metabolites by rats following ingestion is by the feces, amounting to as much as 88% of the administered dose (Barnett and Dorough, 1974). The cis-isomer was excreted to a greater extent than was the trans-isomer (Barnett and Dorough, 1974; Tashiro and Matsumura, 1977). Since more oxychlordane, which is stored in adipose tissue, is formed from trans- than from cis-chlordane, the greater elimination of the cis-isomer is not surprising. Female rats excreted a greater percentage of metabolites in urine than did males (Barnett and Dorough, 1974). Urinary excretion of metabolites by rabbits was much greater than by rats, and in some cases exceeded fecal excretion of metabolites by rabbits (Poonawalla and Korte, 1971; Barnett and Dorough, 1974; Balba and Saha, 1978). Elimination of chlordane or metabolites by the pulmonary route was not observed in rats (Nye and Dorough, 1976). Excretion of chlordane by a 4-year-old girl was nonlinear, with a calculated half-life in serum of 88 days (Aldrich and Holmes, 1969). Urinary excretion declined for 3 days after ingestion, but rose at 35 days possibly as a result of a release of stored chlordane. A half-life of chlordane in serum of 21 days was calculated for a 2-year-old boy who drank chlordane (Curley and Garrettson, 1969). Oxychlordane has been found in the milk of lactating women (Strassman and Kutz, 1977).

Heptachlor and Heptachlor Epoxide. Heptachlor was absorbed from the gastrointestinal tract of rats following intragastric administration, as evidenced by the appearance of residues in blood within 1 hour of oral dosing (Mizyukova and Kurchatov, 1970), and by the pulmonary route by rabbits that breathed ambient air in a heavy pesticide use area, as evidenced by increased levels of heptachlor epoxide in adipose tissue over control levels (Arthur et al., 1975). Absorption of heptachlor by humans can be inferred from many reports of levels of heptachlor epoxide in adipose tissue obtained in autopsy and biopsy samples from the general population (Klemmer et al., 1977; Abbott et al., 1972; 1981; Wassermann et al., 1972; 1974; Curley et al., 1973; Burns, 1974; Greer et al., 1980; Kutz et al., 1979). Heptachlor epoxide has been detected in all monitored tissues of deceased infants, and in cord blood of healthy neonates at levels corresponding to levels in the general adult population (Curley et al., 1969; Zavon et al., 1969; Wassermann et al., 1972, 1974). Following absorption of heptachlor, its metabolite, heptachlor epoxide, is distributed throughout the body of rats and dogs (Mizyukova and Kurchatov, 1970; Radomski and Davidow, 1953). Female rats accumulated 5-10 times as much heptachlor epoxide in adipose tissue as did males following continuous feeding of heptachlor for 8 weeks. Heptachlor epoxide persisted in fat of female rats for 8 weeks and male rats for 6 weeks after treatment was terminated. The maximum dietary level at which fat storage could not be detected was 0.3 mg/kg diet for male rats and 0.1 mg/kg diet for female rats. Heptachlor was metabolized by rats in vivo and in vitro to heptachlor epoxide, which was dechlorinated and hydroxylated to a fecal metabolite (Tashiro and Matsumura, 1978). Heptachlor was also metabolized to 1-hydroxychlordene, which was epoxidized to 1-hydroxy-2,3-epoxychlordene; 1-hydroxy-2,3-epoxychlordene was hydrolysed to 1,2-dihydroxydihydrochlordene. As much as 4 times more heptachlor epoxide was formed in vitro by rat microsomes than by human microsomes; otherwise, metabolism was similar (Tashiro and Matsumura, 1978).

Like chlordane, the major route of heptachlor elimination by rats is by the feces, amounting up to 50% over 10 days of the administered oral dose (Tashiro and Matsumura, 1978). Urinary excretion of metabolites amounted to <5% of the dose. The only information available on human excretion of heptachlor are reports of heptachlor epoxide detected in milk of lactating women (Kroger, 1972; Ritcey et al., 1972; Savage et al., 1973; Bakken and Seip, 1976; Polishuk et al., 1977b; Jonsson et al., 1977; Strassman and Kutz, 1977; Takahashi et al., 1981). This excretion reduced the body burden of heptachlor epoxide in these women but increases the exposure of breast-fed infants.

IV. SOURCE OF HUMAN EXPOSURE - CHLORDANE, HEPTACHLOR AND HEPTACHLOR EPOXIDE

This chapter summarizes available data on use, environmental fate, and occurrence of chlordane, heptachlor and heptachlor epoxide and characterizes their potential for drinking water exposure. A more extensive discussion of the available information on these compounds is presented in the draft document entitled "Occurrence of Pesticides in Drinking Water, Food and Air" (USEPA 1987).

Humans may be exposed to chlordane and heptachlor from a variety of sources, including drinking water, food, ambient and possibly indoor air. Heptachlor epoxide is a degradation product of heptachlor. Humans may be exposed to heptachlor epoxide from water, food and air. This analysis of human exposure is limited to drinking water, food and air. Some individuals (pesticide applicators) will receive additional exposure to chlordane and heptachlor from their occupations. Chlordane is not restricted to licensed applicators and is available to the public. Additional exposure can occur from consumer use. Individual exposure to chlordane, heptachlor and heptachlor epoxide will vary widely based on factors such as where a person lives and travels, and what a person eats. Intake of chlordane, heptachlor and heptachlor epoxide will be affected by age, weight and lifestyle.

The Exposure Estimates section of this chapter presents available information on the range of human exposure to and intake of chlordane, heptachlor and heptachlor epoxide from drinking water, food and air. It is not possible to provide an estimate of the number of individuals experiencing specific exposures from these three sources. However, this section provides insight into the relative contributions of the three sources.

A. Use/Environmental Fate

Chlordane and heptachlor are broad spectrum insecticides which, until the mid-1970's, were widely used as pre-emergent insecticides on many crops. During the mid-1970's, concern over the possible health risks of these two chemicals led to a ban of all their uses other than subsurface ground injection for the control of termites. Heptachlor is often used in conjunction with chlordane for this purpose (USEPA 1983). Heptachlor also retains some additional use on certain crops in Hawaii (Kuch, 1986). In the past, heptachlor has occurred as an impurity in commercial chlordane (USEPA 1980).

Once released into the environment, chlordane is very resistant to chemical and biological degradation. It is highly immobile, and does not leach readily from soil. The average half-life of chlordane in soil is 3.3 years, but some residues have been reported to persist for up to 14 years at detectable levels. The major mechanism for removing chlordane from soil is volatilization (Lyman et al., 1982; Rao and Davidson, 1980). Despite its lack of mobility in the soil column, chlordane's lengthy persistence in the soil may result in a long-term, low-level source of ground water contamination (USEPA 1987).

Chlordane is not removed from natural waters by hydrolysis or biodegradation (Eichelberger and Lichtenberg, 1971, as cited in Callahan et al., 1979). However, chlordane is removed from surface water by adsorption to bottom sediments and by volatilization into the atmosphere. Based on chlordane's physical and chemical properties, it is expected to adsorb to suspended sediments and particulates in surface waters (Lyman et al., 1982).

Heptachlor has environmental fate properties similar to chlordane except that heptachlor is susceptible to hydrolysis. Eichelberger and Lichtenberg (1971, as cited in Callahan et al., 1979), reported that heptachlor can undergo hydrolysis to form 1-hydroxychlordene. This product is then susceptible to biotransformation under either aerobic or anaerobic conditions to produce heptachlor epoxide and other compounds (Miles et al., 1969 and 1971, both cited by Callahan et al., 1979). The mean half-life of heptachlor in soils under aerobic conditions may range from 63 days, as observed in the laboratory, to 426 days, as observed under field conditions (Rao and Davidson, 1980).

Heptachlor epoxide, however, is resistant to hydrolysis and biodegradation, and is very persistent in soils (Callahan et al., 1979).

As with chlordane, both heptachlor and heptachlor epoxide bind strongly to the soil and sediments. Both compounds are therefore resistant to leaching and transport into ground water (USEPA 1987).

In surface waters, heptachlor is susceptible to hydrolysis to 1-hydroxychlordene with an estimated half-life of approximately 1 to 3 days (Demayo, 1972 and Eichelberger and Lichtenberg, 1971, both cited by Callahan et al., 1979). The compound 1-hydroxychlordene degrades in water to form heptachlor epoxide. Both heptachlor and heptachlor epoxide will tend to adsorb onto suspended particulate matter and partition to bottom sediments (Mabey et al, 1981). Under certain conditions, the two compounds may also volatilize.

B. Occurrence

Drinking Water

No national surveys of chlordane, heptachlor and heptachlor epoxide are available to determine the extent of occurrence of these chemicals in public water supplies. A few limited studies have reported detectable levels of the compounds

(USEPA, 1987). These surveys determined that the compounds rarely occurred at detectable levels and when they did occur, the levels were almost always less than 0.1 ug/l.

It is not clear how to relate the results of the above surveys to the current levels of these compounds in drinking water. The surveys generally were designed to detect contaminants resulting from agricultural uses, which were later banned or greatly restricted. EPA has no data on the degree of contamination of drinking water supplies which occurs as a result of current uses of these compounds. However, based on the manner of application (subsoil injection) and the ability of the compounds to bind to soils, contamination would be expected to be limited to ground water supplies. Levels of these compounds, where they occur, are expected to be low.

Diet

Information and estimates on the dietary intake of chlordane, heptachlor and heptachlor epoxide were obtained from the Food and Drug Administration's Total Diet Studies, which are commonly referred to as "Market Basket Surveys" (see Table 1). The dietary intake of heptachlor by 25-30 year old adults is significantly lower than the intake of heptachlor epoxide (FDA, 1986), reflecting the conversion of the compound in the environment.

Table 1

Estimated Daily Intake Levels for 25-30 Year Old Males

Compound	Estimated Adult Intake
Chlordane	0.048 ug/day
Heptachlor	0.007 ug/day
Heptachlor epoxide	0.184 ug/day

The origins of these dietary levels are unclear. There are currently no agricultural uses of chlordane, and heptachlor has only limited agricultural uses. The current dietary levels could be the result of past uses of these compounds. Due to the restricted use of the chemicals, future levels are expected to decline.

Air

No monitoring data are available on the current levels of chlordane, heptachlor and heptachlor epoxide in ambient air. In 1976, a survey of air levels in high use areas reported finding both chlordane and heptachlor in a large percentage of samples taken. The levels found were less than 0.1 ug/m^3. This survey reflects the then widespread agricultural use of chlordane and heptachlor. Because these uses have been discontinued and the current uses have a low potential for volatilization (subsurface injection), the levels reported for the survey are not indicative of current levels of occurrence. Based on the current uses, levels of chlordane, heptachlor and heptachlor epoxide in air are expected to be negligible.

EPA is concerned over the potential occurrences of these compounds in indoor air. Based on the recent experience with radon and other volatile ground water contaminants, EPA suspects that the current practices of subsurface injection for chlordane and heptachlor can result in the contamination of indoor air. No data are available on actual levels.

C. Exposure Estimates

The information that is currently available on the occurrence of chlordane, heptachlor and heptachlor epoxide in the environment and the potential for exposure are insufficient to determine the national distribution of intake by any of the three routes, for any of the compounds. (Table 2). The data reported for drinking water and air exposure were derived from studies conducted during the early to mid-1970's (prior to restrictions) and data for food exposure were collected during the early to mid-1980's; therefore, it is likely that current inhalation and drinking water exposure levels will be much less than the reported levels and that current dietary exposure levels will be similar to the reported levels. Although the exposure to heptachlor is expected to be equally minimal from all three sources, diet is likely to be the greatest source of exposure to heptachlor epoxide.

Because the ranges of potential intake levels from drinking water and air exposure are so wide and varied and because the estimated intake levels provided for food are single values and were collected under different usage conditions it is not possible at this time to generate an accurate estimate of the total exposure. Additional data are needed for estimating the total combined intake from each media.

Table 2

Exposure Estimates

Source	Reported Exposure Levels	Estimated Adult Intake
CHLORDANE		
Drinking Water	<0.1 ug/l	<0.2 ug/day
Diet	--	0.048 ug/day
Air	?*	?
HEPTACHLOR		
Drinking Water	<0.1 ug/l	<0.2 ug/day
Diet	--	0.0007 ug/day
Air	?	?
HEPTACHLOR EPOXIDE		
Drinking Water	<0.1 ug/l	<0.2 ug/day
Diet	--	0.184 ug/day
Air	?	?

*Ambient air levels of the compounds are believed to be very low. Indoor air levels while unknown are expected to be higher.

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V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Effects of Acute Exposure. In the discussion of the health effects of acute exposure of animals to chlordane, heptachlor and heptachlor epoxide, durations of exposure from a single dose up to 15 daily doses will be considered acute.

Chlordane -- Values for the acute oral LD_{50} of chlordane in rats of various strains ranged from 83 mg/kg bw for pure cis-chlordane (Podowski et al., 1979) to 590 mg/kg bw for chlordane of unspecified purity (Ambrose et al., 1953a,b), when delivered by gavage in lipophilic vehicle (Table V-1). The values determined for technical grade chlordane (see Chapter II) by Gaines (1960) of 335 mg/kg bw (confidence limits 299-375 mg/kg bw) for male Sherman rats, and 430 mg/kg bw (391-473 mg/kg bw) for female rats of the same strain, are in good agreement with other values of 311±46 mg/kg bw (Boyd and Taylor, 1969) and 350±22 mg/kg bw (Gak et al., 1976) determined for technical grade chlordane in rats. A slightly lower value for rats (283 mg/kg bw) was reported by Ben-Dyke et al. (1970). Gak et al. (1976) found that mice of an unspecified strain were slightly less sensitive (LD_{50} = 390±35 mg/kg bw) and Golden hamsters were much less sensitive (LD_{50} = 1720±135 mg/kg bw) to chlordane than rats. Chlordane was not as toxic to rats when applied dermally (Gaines, 1960; Ben-Dyke et al., 1970). Harbison (1975) found that neonatal Sprague-Dawley rats were less sensitive than adult male rats to chlordane (analytical grade) intoxication when the insecticide was administered intraperitoneally. Pretreatment of the neonates with phenobarbital, however, reduced the LD_{50} from 1121 to 539 mg/kg bw,

TABLE V-1

Acute LD_{50}s for Chlordane

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	LD_{50} (mg/kg bw)	Comment	Reference
Rat/NR	NR/NR	NR	technical grade	oral	"innocuous solvent"	457	Tremors within 15 minutes, convulsions in 24 hours persisting until 48 hours. Deaths in 6 days.	Lohman, 1951
Rat/ Albino	NR/NR	NR	NR	oral	cottonseed oil	590	NC	Ambrose et al., 1953a,b
Rat/ Sherman	M/70	175 g/90 days	technical grade	oral	peanut oil	335 (299-375)	Survival time: 48 hours-9 days for M and F; tremors, hyperexcitability, irritability and convulsions. Numbers in parentheses are confidence limits.	Gaines, 1960
	F/70	200 g/90 days	technical grade	oral	peanut oil	430 (391-473)		
Rat/ Wistar	M/141	50-60 g/ 56 days	technical grade	oral	cottonseed oil	311 ± 46	Death in 42 ± 18 hours. Clinical symptoms: stimulation of CNS irritability; phonation; piloerection; tremors and convulsions alternating with depression; hyporeflexia; lethargy; diarrhea; diuresis; anorexia; oligodipsia; hypothermia; low bw. At necropsy, local gastroenteritis, nephritis, hepatitis, adrenal and thymus stress reactions, vascular congestion, dehydration, loss of organ weight. LD_{10} = 237 mg/kg bw - estimated max. LD_{50} = 386 mg/kg bw - estimated max.	Boyd and Taylor, 1969
Rat/ Charles River	M/6-10/ group	150 g/6 weeks	cis-chlordane 99.9+%	oral	corn oil	83	Deaths in 24 hours.	Podowski et al., 1979
Rat/NR	NR/NR	NR	NR	oral	olive oil	350 ± 22	NC	Gak et al., 1976
Mouse/NR	NR/NR	NR	NR	oral	olive oil	390 ± 35	NC	Gak et al., 1976
Hamster/ Golden	NR/NR	NR	NR	oral	olive oil	1720 ± 135	NC	Gak et al., 1976

TABLE V-1 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	LD_{50} (mg/kg bw)	Comment	Reference
Rat/NR	NR/NR	NR	commercial formulation	oral	NR	283	NC	Ben-Dyke et al., 1970
Rat/NR	NR/NR	NR	commercial formulation	dermal	NR	>600	NC	Ben-Dyke et al., 1970
Rat/ Sherman	M/70	175 g/90 days	technical grade	dermal	xylene	840 (750-941)	Survival time: 72 hours - 9 days	Gaines, 1960
	F/70	200 g/90 days	technical grade	dermal	xylene	690 (580-821)	96 hours - 25 days	
	F/70	200 g/90 days	technical grade	dermal	undiluted	530 (431-652)	120 hours - 15 days Numbers in parentheses are confidence limits.	
Rat/ Sprague-Dawley	M/F 10-20/ group	Neonates 2-5 days	analytical grade	i.p.	corn oil	1121 (984-1289)	Pretreatment with phenobarbital (40 mg/kg bw x 3) reduced the LD_{50} to 539 mg/kg bw (428-666). Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/ Sprague-Dawley	M 10-20/ group	120-150 g/ adult	analytical grade	i.p.	corn oil	343 (250-451)	Numbers in parentheses are confidence limits.	Harbison, 1975
Mouse/ Swiss-Webster	M/NR	20 g	cis-chlordane	i.p.	dimethyl sulfoxide	30	NC	Ivie et al., 1972
			trans-chlordane	i.p.		130		

NC = No comment

NR = Not reported

i.p. = intraperitoneal

by enhancing the ability of neonates to metabolize chlordane to its toxic intermediate. Pure cis-chlordane was more toxic than pure trans-chlordane to male Swiss-Webster mice when administered intraperitoneally (Ivie et al., 1972). The pure cis-isomer was also more toxic to rats than was technical grade chlordane. Further details of the LD_{50} determinations are presented in Table V-1.

Symptoms of acute intoxication include CNS stimulation, as evidenced by irritability, tremors and convulsions (Boyd and Taylor, 1969; Stohlman et al., 1950; Hyde and Falkenberg, 1976). Boyd and Taylor (1969) described a wide range of CNS disturbances, including phonation; piloerection; tremors and convulsions alternating with lethargy; diarrhea; and food and water refusal. Necropsy of rats revealed vascular congestion, nephritis, hepatitis and decreased organ weight.

Other studies of acute exposure, other than LD_{50} determinations, are described in Table V-2. Stohlman et al. (1950) have conducted an extensive study on dose response, vehicle effect and effect of route of administration of chlordane (described as "specially purified") on mortality of rats and rabbits. Chlordane was more toxic when administered orally to rats and rabbits in Tween-20 than in olive oil, as evidence by greater incidence of mortality occurring at shorter periods following treatment. No deaths in rats occurred at a single oral dose of 100 mg/kg bw in Tween-20, although the same dose resulted in three deaths in eight rabbits within 2.5 hours. Multiple oral doses of 10 mg/kg bw/day in olive oil for 16 days resulted in 2 deaths among five rabbits. Increased mortality in rats after intraperitoneal administration and in rabbits after intravenous administration of chlordane was also observed (see Table V-2) (Stohlman et al., 1950).

TABLE V-2

Acute Toxicity of Chlordane

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	NR/10	130-400 g	octa-klor ("specially purified")	oral	Tween-20	0 mg/kg bw	single dose	0/10 deaths	Stohlman et al., 1950
	6					100 mg/kg bw		0/6 deaths	
	13					200 mg/kg bw		7/13 deaths (53%); tremors and convulsions.	
	21					300 mg/kg bw		13/21 deaths (62%); tremors and convulsions.	
	19					1000 mg/kg bw		18/19 deaths (95%); tremors and convulsions.	
Rat/NR	NR/10	134-400 g	octa-klor ("specially purified")	oral	olive oil	200 mg/kg bw	single dose	1/10 deaths (10%) in 30 days	Stohlman et al., 1950
	10					250 mg/kg bw		6/10 deaths (60%) in 1-7 days	
	10					500 mg/kg bw		8/10 deaths (80%) in 2-5 days No mention of controls was made for this series.	
Rats/ Wistar	M/4/ groups	200 g	cis-chlordane	oral	corn oil	0, 200 mg/kg bw	single dose	Fasting blood glucose increased to 147% of control; decreased liver glycogen (64%), increased serum urea (125%); increased activities of hepatic pyruvate carboxylase (183%), fructose-1,6-diphosphatase (236%) and glucose-6-phosphatase (182%). Increased kidney cortical enzymes as above: 156%, 178%, 189% and 175%, respectively. Increased liver and kidney basal and fluoride-stimulated adenyl cyclase, and cyclic AMP. All the changes were significantly different from control levels ($p<0.05$).	Kacew and Singhal, 1973 Singhal and Kacew, 1976

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rabbit/NR	NR/6	1.6-3.3 kg	octa-klor ("specially purified")	oral	Tween-20	0 mg/kg bw	single dose	0/6 no effect	Stohlman et al., 1950
	8				Tween-20	100 mg/kg bw		3/8 deaths (38%) 2-2.5 hours; dyspnea, tremors, convulsions.	
	6				Tween-20	200 mg/kg bw		5/6 (83%) deaths 2-4 hours, dyspnea, tremors, convulsions.	
	13				olive oil	300 mg/kg bw		5/13 (38%) deaths 6-22 days.	
	9				olive oil	400 mg/kg bw		7/9 (78%) deaths 4-31 days.	
	11				olive oil	600 mg/kg bw		8/11 (73%) deaths 2 hours to 8 days; dyspnea, tremors, convulsions.	
	7				olive oil	1000 mg/kg bw		7/7 (100%) deaths 3 hours to 4 days; dyspnea, tremors, convulsions.	
Mouse/ CD-1	M-F/NR	60-70 days	technical grade	oral	corn oil	100 mg/kg bw 300 mg/kg bw 1000 mg/kg bw	single dose	No symptoms Mild hypoactivity-no deaths 100% mortality	Arnold et al., 1977
Mouse/ CD-1	M-F/NR	60-70 days	HCS-3260 (98+%)	oral	corn oil	30 mg/kg bw 100 mg/kg bw 300 mg/kg bw 1000 mg/kg bw	single dose	No symptoms No symptoms Intermittent tremors and ataxia followed by death. Intermittent tremors and ataxia followed by death.	Arnold et al., 1977
Rat/NR	M/12	50-60 g	octa-klor ("specially purified")	oral	diet	0.1%, 1000 mg/kg diet	10 days	100% mortality	Stohlman et al., 1950
Rat/ Albino	NR/5	233-335 g	NR	oral	cottonseed oil	0, 6.25, 12.5, 25, 50, 100, 200 mg/kg bw/day	15 days	No observed symptoms of toxicity except for slight histologic changes in the liver of group exposed to <25 mg/kg/day. In the next highest group, 2 of the 5 animals died.	Ambrose et al., 1953a,b
Rat/ Wistar	M/ 6/group	80-130 g	technical grade	oral	diet	0, 5, 10, 20 or 50 mg/kg diet	14 days	Dose-related enzyme induction of liver aniline hydroxylase, aminopyrine dimethylase and hexabarbital oxidase, significant at 10-50 mg/kg diet, 10-50 mg/kg diet and 20-50 mg/kg diet, respectively ($p<0.025$). No-effect level - 5 mg/kg diet, lowest-effect level - 10 mg/kg diet.	Den Tonkelaar and Van Esch, 1974

TABLE V-2 (cont.)

Species/Strain	Sex/Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rabbit/NR	NR/5	1.6-3.3 kg	octa-klor ("specially purified")	oral	olive oil	5	0-15 days	0/5- no deaths until 53 days of feeding.	Stohlman et al., 1950
	5					10		0/5- deaths at 14 days, 2 deaths at 16 days.	
	4					15		4/4 deaths - 13 days	
	5					20		5/5 deaths - 10 days	
	14					50		14/14 deaths - 15 days	
	12					100		12/12 deaths - 9 days	
	6					200 mg/kg bw/day		6/6 deaths - 8 days	
Rat/NR	NR/NR	NR	cis-chlordane	NR	NR	200 mg/kg bw	single dose	Slight tremors, paralysis of hind legs, convulsions, hypothermia	Hrdina et al., 1974
Mouse/Swiss	F/20/group	NR	technical grade	inhalation	air	current of air (18 mℓ/sec) passed through 105 mℓ of chlordane in a saturation train	continuous for 14 days	No symptoms of toxicity for either grade of chlordane. Histologically, there was reticulation and oxyphilia of liver cytoplasm, congestion and cell proliferation of bronchiole lining.	Ingle, 1953
			AAEE reference standard	inhalation	air				
Rat/NR	NR/10	130-400 g	octa-klor ("specially purified")	i.p.	none	160 mg/kg bw	single dose	3/10 (30%) deaths in 9-10 days	Stohlman et al., 1950
	10				none	240 mg/kg bw		8/10 (80%) deaths in 5-7 days	
	7				none	400 mg/kg bw		6/7 (86%) deaths in 2-6 days Pulmonary congestion and hemorrhaging.	
	19				Tween-20	0 mg/kg bw	single dose	2/19 (11%) deaths in 7-9 days; adhesions and exudates.	
	10				Tween-20	50 mg/kg bw		1/10 (10%) deaths in 13 days	
	20				Tween-20	100 mg/kg bw		3/20 (15%) deaths in 1.5 hours-6 days.	
	20				Tween-20	200 mg/kg bw		15/20 (75%) deaths in 40 minutes-6 days. Tremors, convulsions.	
	10				olive oil	100 mg/kg bw	single dose	1/10 (10%) deaths in 8 days	
	10				olive oil	200 mg/kg bw		4/10 (40%) deaths in 2-3 days	
	10				olive oil	300 mg/kg bw		7/10 (70%) deaths in 3-6 days	

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/ Sprague-Dawley	M/2	255-275 g	technical grade	i.p.	3% Tween-80 isotonic saline	350 mg/kg bw	single dose	Deaths within 1 hour; mild tremors and disorientation in minutes; hypersensitivity progressing to clonic and recurrent ataxia, muscular rigidity, tetanic paralysis, hyperpnea; changes in EEG.	Hyde and Falkenburg, 1976
Mouse/ CD-1	M-F/NR	60-70 days	technical grade	i.p.	corn oil	100 mg/kg bw	single dose	No symptoms of toxicity	Arnold et al., 1977
						300 mg/kg bw		Mild hypoactivity, 50% mortality	
						1000 mg/kg bw		100% mortality	
Mouse/ CD-1	M-F/NR	60-70 days	HCS-3262	i.p.	corn oil	30 mg/kg bw	single dose	No symptoms of toxicity	Arnold et al., 1977
						100 mg/kg bw		No symptoms of toxicity	
						300 mg/kg bw		Intermittent tremors and ataxia among males.	
						1000 mg/kg bw		Intermittent tremors and ataxia among males, no mortality.	
Mouse/ Balb/ c Cr1	F/60/ group	neonate	cis-chlordane 100%	s.c.	sesame oil	0 0.075 mg/mouse	3 injections, 1 each on days 2, 3 and 4 of life	Significant differences from control: delayed eye opening (p<0.001); delayed vaginal opening (p<0.001), decreased bw at 4, 6 and 8 weeks.	Talamantes and Jang, 1977
						0.15 mg/mouse		Decreased bw at 4, 6 and 8 weeks, increased ovary weight (p<0.05).	
			trans-chlordane 99.3%	s.c.	sesame oil	0.075 mg/mouse	3 injections, 1 each on days 2, 3 and 4 of life	Significantly delayed eye opening (p<0.01), decreased bw at 4, 6, 8 and 10 weeks; decreased pituitary weight (p<0.05).	
						0.15 mg/mouse		Delayed vaginal opening (p<0.05), decreased bw at 4 weeks, decreased pituitary weight (p<0.02), increased ovary weight (p<0.02).	

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Gerbil/ Indian desert	M/20/ group	70-90 g/adult	technical grade	i.m.	petroleum ether	0, 25, 50, 75 mg/kg bw	single dose	Dose-related observed hyperglycemia maximal 1 hour after treatment: blood glucose levels declined to control levels 1 week after treatment.	Saxena and Karel, 1976
Rabbit/NR	NR/15	1.6-3.3 kg	octa-klor ("specially purified")	i.v.	Tween-20	0 mg/kg bw	single dose	0/15 deaths	Stohlman, et al., 1950
	7				Tween-20	10 mg/kg bw		1/7 (14%) deaths in 14 hours	
	23				Tween-20	20 mg/kg bw		17/23 (74%) deaths in 7 minutes to 4 days.	
	10				Tween-20	30 mg/kg bw		10/10 (100%) deaths in 7 minutes to 2 days.	
	3				Tween-20	40 mg/kg bw		3/3 (100%) deaths in 15-30 minutes, dyspnea, tremors and convulsions.	

NR = Not reported

i.m. = intramuscular

i.p. = intraperitoneal

i.v. = intravenous

s.c. = subcutaneous

Ambrose et al. (1953a) found no symptoms of toxicity except for intracytoplasmic bodies in the liver cells in all albino rats (groups of 5) treated orally for 15 days with 6.25 or 25 mg chlordane/kg bw/day in cottonseed oil. At doses of 50, 100 or 200 mg/kg bw/day, 2, 5 and 5 animals/group died. A single oral dose of 200 mg/kg bw cis-chlordane in corn oil resulted in significantly elevated liver and kidney gluconeogenic enzymes when compared with controls (Kacew and Singhal, 1973). Similar results were observed in gerbils treated intramuscularly with technical grade chlordane (Saxena and Karel, 1976).

Single oral doses of technical grade chlordane or of HCS-3260 (see Chapter III) to CD-1 mice resulted in no signs of toxicity at 30-100 mg/kg bw doses (Arnold et al., 1977). Mild hypoactivity was observed at 300 mg technical grade chlordane/kg bw and 100% mortality at a dose of 1000 mg/kg bw. Similar doses of HCS-3260 resulted in tremors, ataxia and death. Intraperitoneal doses of these preparations produced results similar to those observed after oral administration. Den Tonkelaar and Van Esch (1974) determined the dose response of technical grade chlordane administered in the diet for 14 days to groups of six male Wistar rats. Significantly elevated ($p<0.025$) activities of aniline hydroxylase and aminopyrine demethylase occurred at a chlordane concentration of ≥ 10 mg/kg diet and of hexobarbital oxidase at ≥ 20 mg/kg diet. Enzyme activities at 5 mg/kg diet levels were not significantly different from control values. Continuous inhalations for 14 days of technical grade or AAEE reference standard chlordane (see Table V-2 for exposure) produced no outward signs of toxicity in Swiss mice; however, mild histological changes were seen in liver and lung tissues at necropsy (Ingle, 1953).

Neonatal female rats treated with subcutaneous injections of cis- or trans-chlordane (0.075 or 0.15 mg/mouse) on days 2, 3 and 4 of life had significantly delayed onset of eye opening and vaginal opening, decreased bw, decreased pituitary weight and increased ovary weight when compared with untreated controls (Talamantes and Jang, 1977). A dose-response relationship for these effects was not demonstrated for either isomer.

Heptachlor and Heptachlor Epoxide -- Acute oral LD_{50} values of heptachlor administered in lipophilic vehicles to rats, mice and hamsters are in good agreement (Table V-3). The range of values for rats was 71 g/kg bw for male Charles River rats (Podowski et al., 1979) to 162 mg/kg bw for female Sherman rats (Gaines, 1960). For male Sherman rats, the LD_{50} was 100 mg/kg bw. Gak et al. (1976) found that rats and hamsters were equally susceptible (LD_{50} = 105±12 mg/kg bw and 100±11 mg/kg bw, respectively). A value of 40 mg/kg bw in rats was reported for the less pure commercial formulation (Ben-Dyke et al., 1970). Gak et al. (1976) reported that heptachlor was slightly more toxic to mice (LD_{50} = 70±7 mg/kg bw). Heptachlor applied dermally was less toxic than when administered orally to rats (Gaines, 1960; Ben-Dyke et al., 1970). Harbison (1975) found that upon intraperitoneal administration, analytical grade heptachlor was 7.5 times less toxic to neonatal Sprague-Dawley rats than to adult males. Pretreatment with phenobarbital, however, reduced the neonatal LD_{50} from 531 to 133 mg/kg bw by enhancing the neonates' ability to bioactivate the insecticide to the more toxic heptachlor epoxide. The acute oral LD_{50} of heptachlor epoxide in adult rats was ~60 mg/kg bw (NAS, 1977; Sperling and Ewinike, 1969; Podowski et al., 1979). The intraperitoneal LD_{50} of heptachlor epoxide in mice was 18 mg/kg bw (Ivie et al., 1972). Further details of the LD_{50} determinations are presented in Table V-3.

TABLE V-3

Acute LD_{50}s for Heptachlor and Heptachlor Epoxide

Species/ Strain	Sex/ Number	Weight/Age	Compound and/or Purity	Route	Vehicle	LD_{50} (mg/kg bw)	Comment	Reference
Rat/NR	NR/NR	NR	"pure" heptachlor	oral	"innocuous solvent"	90	Tremors and convulsions, deaths occurred up to 6 days.	Lehman, 1951
Rat/ Sherman	M/60	175 g/90 days	technical heptachlor	oral	peanut oil	100 (74-135)	Tremors, hyperexcitability, irritability and convulsions Survival time: 6 hours - 7 days	Gaines, 1960
	F/90	200 g/90 days				162 (140-188)	Survival time: 5 hours - 11 days numbers in parentheses are confidence limits.	
Rat/NR	M/NR	adult	heptachlor purity NR	oral	NR	112	NC	Sperling and Ewinke, 1969
Rat/ Charles River	M 6-10/ group	150 g/6 weeks	heptachlor 99.9+%	oral	corn oil	71	Deaths occurred in 24 hours	Podowski et al., 1979
Rat/NR	NR/NR	NR	heptachlor purity NR	oral	olive oil	105 ± 12	NC	Gak et al., 1976
Mouse/NR	NR/NR	NR	heptachlor purity NR	oral	olive oil	70 ± 7	NC	Gak et al., 1976
Hamster/ Golden	NR/NR	NR	heptachlor purity NR	oral	olive oil	100 ± 11	NC	Gak et al., 1976
Rat/NR	NR/NR	NR	heptachlor commercial formulation	oral	NR	40	NC	Ben-Dyke et al., 1970
Rat/NR	NR/NR	NR	heptachlor commercial formulation	dermal	NR	195-250	NC	Ben-Dyke et al., 1970

TABLE V-3 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Compound and/or Purity	Route	Vehicle	LD_{50} (mg/kg bw)	Comment	Reference
Rat/ Sherman	M/100	175 g/90 days	technical heptachlor	dermal	xylene	195 (119-320)	Tremors, hyperexcitability, irritability and convulsions Survival time: 43 hours - 10 days	Gaines, 1960
	F/60	200 g/90 days				250 (200-313)	Tremors, hyperexcitability, irritability and convulsions Survival time: 96 hours - 13 days Numbers in parentheses are confidence limits.	
Rat/ Sprague-Dawley	M/F 10-20/ group	neonates 2-5 days	heptachlor analytical grade	i.p.	corn oil	531 (311-1020)	Pretreatment with phenobarbital (40 mg/kg bw x 3) i.p. reduced LD_{50} to 133 mg/kg bw. Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/ Sprague-Dawley	M/ 10-20/ group	120-150 g/ adult	heptachlor analytical grade	i.p.	corn oil	71 (41-103)	Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/NR	M/NR	adult	heptachlor epoxide	oral	NR	62	NC	Sperling and Ewinike, 1969
Rat/ Charles River	M/ 6-10/ group	150 g/6 weeks	heptachlor epoxide	oral	corn oil	60	Deaths in 24 hours	Podowski et al., 1979
Rat/NR	M/NR	NR	heptachlor epoxide	oral	NR	46.5	NC	NAS, 1977
	F/NR					61.3		
Mouse/ Swiss-Webster	M/NR	20 g	reference heptachlor epoxide	i.p.	dimethyl sulfoxide	18	NC	Ivie et al., 1972

NC- No comment

NR- Not reported

i.p.- intraperitoneal

Other acute toxicity data for heptachlor and heptachlor epoxide are presented in Table V-4. Symptoms of intoxication include tremors, convulsions, paralysis and hypothermia (Hrdina et al., 1974; Yamaguchi et al., 1980). Oral doses of heptachlor have resulted in significantly elevated levels of serum GPT and serum aldolase coincident with histologically observed liver damage (Krampl, 1971). These effects were observed in female Wistar rats given a single oral dose of 98% pure heptachlor of 60 mg/kg bw or repeated oral doses of 7 or 12 mg/kg bw/day for up to 28 days. At 28 days, the serum levels of GPT and aldolase had returned to control levels, and damage to the liver was less severe. It is possible that the rats were developing a tolerance to heptachlor. At a high dose (200 mg/kg bw orally), which is above the oral LD_{50}, significantly increased ($p<0.05$) activities of gluconeogenic enzymes were observed in male Wistar rats (Kacew and Singhal, 1973; Singhal and Kacew, 1976).

A single day or 5-7 days of dietary administration of heptachlor (96%) at a level of 10 mg/kg diet to rats resulted in alterations in liver function, as evidenced by changes in blood glucose, liver glycogen, GPT levels, acid and alkaline phosphatase activities, etc. (see Table V-4 for other parameters and directions of changes) (Enan et al., 1982). Single oral or intraperitoneal doses (30 or 100 mg/kg bw) of a heptachlor:heptachlor epoxide (27:75) preparation to CD-1 mice resulted in moderate to severe hypoactivity, ruffled fur and "partial mortality" (Arnold et al., 1977). At low dietary levels of >99% pure heptachlor (2-50 ppm or mg/kg diet) for 14 days, dose-related induction of hepatic aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase was observed in rats (Den Tonkelaar and Van Esch, 1974). Details of these studies are given in Table V-4.

TABLE V-4

Acute Toxicity of Heptachlor and Heptachlor Epoxide

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/ Wistar	F/24/ group	200-300 g	heptachlor 98%	oral	vegetable oil	0, 60 mg/kg bw	single dose	Subgroups were sacrificed at 2, 24 and 72 hours. Significant changes ($p<0.05-0.001$): elevated liver GPT and aldolase at 2 hours, decreased liver GPT and aldolase at 72 hours. Elevated serum GPT at 72 hours and serum aldolase at 24 and 72 hours. Changes coincided with slight to moderately severe histological liver damage.	Krampl, 1971
Rat/NR	NR/NR	NR	heptachlor	NR	NR	200 mg/kg bw	single dose	Slight tremors, hind leg paralysis, convulsions and hypothermia.	Hrdina et al., 1974
Rat/ Wistar	M/4/ group	200 g/ adult	heptachlor	oral	corn oil	0, 200 mg/kg bw	single dose	Increased fasting, blood glucose level (148% of control); decreased concentration of liver glycogen in 1 hour (59%); increased hepatic pyruvate carboxylase (199%), phosphoenolpyruvate carboxykinase (200%), fructose-1,6-diphosphatase (239%) and glucose-6-phosphatase (158%); increased kidney cortical enzymes as above, 167%, 178%, 188% and 138%, respectively. Increased liver and kidney basal and fluoride stimulated adenyl cyclase and cyclic AMP. All changes significantly different from control ($p<0.05$).	Kacew and Singhal, 1973 Singhal and Kacew, 1976
Rat/NR	F/5	100-150 g	heptachlor 96%	oral	diet	0 and 10 mg/kg diet	1 day of feeding	No effect on RBC count, significantly increased WBC count ($p<0.05$), significantly elevated serum bilirubin ($p<0.05$) decreased hepatic lipid content (n.s), increased blood urea (n.s.) and increased serum cholesterol (n.s.), no change in blood glucose or hepatic protein content, decreased liver glycogen content (n.s.), decreased GPT (n.s.), increased GOT (n.s.), increased alkaline phosphatase ($p<0.05$) and decreased acid phosphatase ($p<0.05$) when compared with controls.	Enan et al., 1982

TABLE V-4 (cont.)

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	F/5	100-150 g	heptachlor 96%	oral	diet	0 and 10 mg/kg diet	5 or 7 days* of feeding	No effects on RBC count, increased WBC and serum billirubin ($p<0.05$), no change in hepatic lipid content; increased blood urea ($p<0.05$), no change in serum cholesterol, increased blood glucose ($p<0.05$), decreased liver glycogen content ($p<0.05$). Increased GPT (n.s.), decreased GOT (n.s.), increased acid and alkaline phosphatase ($p<0.05$), increased liver, heart and spleen weights (significance NR).	Enan et al., 1982
Rat/ Wistar	F/24/ group	200-300 g	hepatchlor 98%	oral	vegetable oil	0, 7 and 12 mg/kg bw/day	3-28 days	Subgroups of rats (6) were sacrificed at 3, 7 and 14 days. Significant changes ($p<0.05$-0.001): at 7 mg/kg day - elevated serum GPT and aldolase at 3, 7 and 14 days; reduced liver GPT and aldolase at 7 days, changes coincided with moderately severe liver damage; at 12 mg/kg/day - elevated serum GPT at 3, 7 and 14 days and aldolase at 7 and 14 days. Reduced liver GPT and aldolase at 7 and 14 days. Changes corresponded to moderately severe to severe histological liver damage.	Krampl, 1971
Rat/ Wistar	M/6/ group	80-130 g	heptachlor >99%	oral	diet	0, 2, 5, 10, 20 or 50 mg/kg diet	14 days	Dose-related enzyme induction of liver aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase, significant at 2-50 mg/kg diet; 5-50 mg/kg diet and 5-50 mg/kg diet, respectively ($p<0.025$). No-effect level <2 mg/kg diet, lowest-effect level- 2 mg/kg diet.	Den Tonkelaar and Van Esch, 1974
Mouse/ CD-1	M-F/NR	60-70 days	heptachlor: heptachlor epoxide (25:75)	oral or i.p.	corn oil	30 mg/kg bw	single dose	Moderate hypoactivity and ruffled fur, 25% mortality.	Arnold et al., 1977
						100 mg/kg bw		Severe hypoactivity and "partial mortality"	

TABLE V-4 (cont.)

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	NR/NR	NR	heptachlor epoxide	NR	NR	100 mg/kg bw	single dose	Slight tremors, hind leg paralysis, convulsions, hypothermia.	Hrdina et al., 1974
Rat/ Sprague-Dawley	M/2/ group	160-200 g	hepatchlor expoxide	i.p.	NR	0, 200 mg/kg bw	single dose	Violent convulsions 5 hours after treatment; animals were sacrificed - there was inhibition of brain synaptosomal Ca^{2+}-Mg^{2+} ATPase (see Chapter VII).	Yamaguchi et al., 1980

*The rats were maintained on the test diet for 5 days a week for 4 weeks. It was not clear whether rats were sacrificed after the 7th day of feeding, on the 9th day of the experiment or after the 5th day of feeding on the 7th day of the experiment.

NR- Not reported

i.p.- intraperitoneal

n.s.- not significant

GPT- glutamate pyruvate transaminase

GOT- glutamate oxaloacetate transaminase

Effects of Subchronic and Chronic Exposures. The majority of the available studies on the effects of subchronic and chronic exposure to chlordane, heptachlor and heptachlor epoxide have been conducted by incorporating the insecticides into the diets of laboratory rats and mice. Many of the long-term studies were designed primarily to assess the carcinogenicity of these compounds; however, some data on toxicity were available.

Chlordane --

Subchronic Dietary Exposure. Table V-5 summarizes the studies discussed in this section.

A preliminary subchronic study was conducted to determine the dose levels of chlordane to be administered to Osborne-Mendel rats and B6C3F$_1$ mice for carcinogenicity testing (NCI, 1977a). Male and female rats, five of each sex per group, were administered diets containing chlordane (analytical grade 94.8%) in concentrations of 0-1600 ppm (1600 mg/kg diet) for 42 days. The rats were then maintained on chlordane-free diets for 2 weeks. The only criteria for toxicity were food consumption, body weight gain and mortality. No effects on body weight gain were observed at <400 mg/kg diet. Diets containing 800 mg/kg diet resulted in deaths of four females. A dietary level of 1600 mg/kg diet resulted in 100% mortality.

Groups of five male and five female mice received diets containing 0-320 ppm (320 ppm mg/kg diet) chlordane for 42 days, followed by 14 days observation (NCI, 1977a). No deaths occurred at <80 mg/kg diet. Diets of 160 mg/kg diet resulted in two deaths among males, none in females. All females

TABLE V-5

Effects in Rats and Mice of Subchronic and Chronic Dietary Exposure to Chlordane

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet)*	Duration	Effect	Reference
Rat/ Osborne-Mendel	M-F/5/sex/ group	NR	analytical grade chlordane, 94.8% (71.7% cis-, 23.1% trans-chlordane)	0, 50, 100, 200, 400, 800, 1600	42 days on test diet, 14 days of observation	No effects or bw gain at <400 mg/kg diet. At 800 mg/kg diet, 4/5 females died, no males died, but bw gain was reduced during first week only. At 1600 mg/kg diet, all died.	NCI, 1977a
Mouse/ $B6C3F_1$	M-F/5/sex/ group	NR	analytical grade 94.8%	0, 20, 40, 80, 160, 320	42 days on test diet, 14 days of observation	No deaths at <80 mg/kg diet; at 160 mg/kg diet, 2/5 males, 0/5 females died. At 320 mg/kg diet 2/5 males, 5/5 females died.	NCI, 1977a
Rat/ Sprague-Dawley	M/42/group	91 days	technical grade	0 and 19.5 mg/kg bw/day based on food consumption data	90 days	No effect on food consumption when compared with controls. Decreased mean weekly bw gain compared with controls. No significant change in testicular and ventral prostate weight. Significant increased nuclear (642%) but not cytoplasmic androgen receptor site content of ventral prostate ($p<0.05$), decreased ventral prostate protein content (13% of control), RNA (35%) and DNA content (25%).	Shain et al., 1977
Rat/NR	M-F/NR	NR	technical grade or pure trans-chlordane	various dietary levels	91 days	Dose-related increased phosphorothioate detoxication, increased activities of O-dimethylase and N-dimethylase over controls. The greatest increase was observed during the first week of treatment. Male rats were more sensitive than female rats. The NOEL for enzyme induction was 1 ppm (1 mg/kg diet).	Kinoshita and Kempf, 1970
Rat/NR	M-F/NR	NR	technical grade	0 and 1.2 mg/kg bw/day based on food	5 months	2 control rats died. No histopathological damage to lungs, heart, stomach, liver, kidney, spleen and testes. One treated rat had adenocarcinoma unrelated to treatment.	De Long and Ludwig, 1954

TABLE V-5 (cont.)

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet)*	Duration	Effect	Reference
Mouse/ C57Bl 6N	M/200 controls Number of test mice NR	5 weeks	90% chlordane; 10% heptachlor	0	>36 weeks	0% "benign proliferative lesions" or carcinomas in liver.	Becker and Sell, 1979
				25		2% had benign proliferative lesions (clearly demonstratable foci, areas or nodular lesions which demonstrated neither microinvasion nor cytological atypia).	
				50		7% had benign proliferative lesions. It was unclear how many of the treated mice with benign proliferative lesions also had carcinomas or elevated α-fetoprotein levels. It was stated that of the 12 livers with benign proliferative lesions in the absence of carcinoma, none had elevated α-fetoprotein levels.	
Rat/NR	M/NR	NR	refined technical grade	0, 10, 20, 40, 80, 160, 320, 640 and 1280	400 days	Dose-related growth retardation; occasional liver pathology at 40 mg/kg; enlarged livers and liver pathology at ≥80 mg/kg; increased mortality at ≥640 mg/kg	Ambrose et al., 1953a,b
	F/NR	NR	refined technical grade	0, 10, 20, 40, 80, 160, 320, 640 and 1280	400 days	Growth retardation at ≥320 mg/kg; enlarged livers and liver pathology at 80 mg/kg; increased mortality at ≥640 mg/kg	
Mouse/ CD-1	M-F/ 100/ sex/ group	6 weeks	analytical reference standard (technical grade)	0, 5, 25 and 50	18 months interim kill at 6 months of 10 mice/ sex/group	No effect on bw gain consumption was observed. There was a dose-related increased mean liver weight in treated males and females at 6 months and at 18 months. These increases were significant for all treated female groups, for the 50 mg/kg diet males at 6 months and for the 25 and 50 mg/kg diet males at 18 months. Survival was 14% for males and 24% for females at the highest dose-level and 51-73% for all other groups including controls, neoplastic lesions of the liver were observed (see Carcinogenicity Section). Hepatocytomegaly was observed in a dose-related increased incidence for all treated males and females. The incidence of hyperplastic nodules was dose-related at the 2 highest levels for males and females and significant ($p<1\times10^{[illegible]}$). Re-evaluation of slides by other pathologists resulted in decreased incidences of nodules in favor of greater incidence of carcinomas (see Carcinogenicity Section).	Epstein, 1976 (review of IRDC, 1973a)

TABLE V-5 (cont.)

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet)*	Duration	Effect	Reference
Rat/ Osborne-Mendel	M/10 matched, 60 pooled controls. 50 treated/ group	35 days	analytical grade, 94.0%	0 TMA 203.5, 407.0	109 weeks 80 weeks on test diet, 29 weeks observation	Mean bw of high-dose males and females were consistently decreased from controls. Most high-dose females had tremors at 44 weeks. All treated groups had clinical symptoms of toxicity throughout the study becoming progressively worse with time (loss of bw, rough and discolored hair, palpable masses and tumors). Among females, there was a significant ($p=0.003$) dose-related increase in mortality. No significant difference in mortality was observed for males. Histologic signs of aging were observed with equal frequency in control and treated rats. Neoplastic lesions were observed (see Carcinogenicity Section). Hyperplastic lesions were observed in thyroid glands of rats of all groups. These were follicular- and C-cell hyperplasia.	NCI, 1977a
	F/10 matched, 60 pooled controls. 50 treated/ group	35 days		0 TMA 120.8, 241.5	109 weeks 80 weeks on test diet, 29 weeks observation		
Mouse/ B6C3F1	M/20 matched, 100 pooled, controls. 50 treated/ group	35 days	analytical grade 94.0%	0 TMA 29.9, 56.2	91 weeks 80 weeks on test diet, 10 weeks observation	No significant difference in bw gain between treated males and females and control males and females. High-dose males and females had tremors at 20 weeks. A few mice had general or local hair loss and a hunched appearance at 50 weaks. Abdominal distention, more prevalent in females, was observed in all groups. Significant differences in mortality were observed between low-dose and control males ($p<0.02$) and between high-dose and control males ($p=0.01+$). No difference in mortality among females. Histological signs of aging were seen in all groups. Neoplastic lesions were observed (see Carcinogenicity Section). Hepatocytomegaly and hyperplastic lesions were observed infrequently in all groups.	NCI, 1977a
	F/20 matched, 80 pooled controls. 50 treated/ group	35 days		0 TMA 30.1, 63.8	91 weeks 80 weeks on test diet, 10 weeks observation		
Rat/F-344	80/sex/group		technical grade	0, 1, 5 and 25 mg/kg diet calculated to be 0, 0.045, 0.229 and 1.175 mg/kg/day	130 weeks	Significant dose-related hepatocellular necrosis in males and hepatocellular swelling in high-dose females. Liver adenomas in high-dose males.	Yonemura et al., 1983

TABLE V-5 (cont.)

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet)*	Duration	Effect	Reference
Rat/ Osborne-Mendel	M-F/20 of each sex/ group	50-55 g	technical	5	up to 2 years	No effect on food consumption, growth rate or mortality, no symptoms of toxicity, no gross or histopathological changes in liver, lungs, kidneys, pancreas, stomach, adrenals, thyroid, thymus, lymph, testes, ovaries, heart and spleen.	Ingle, 1952
				10		Essentially the same as at 5 mg/kg diet; however, upon necropsy, minor liver damage (occasional hypertrophic cells, minor bile duct proliferation).	
				30		No effect on food consumption, growth rate, mortality or organ weights. Tremors could be induced after 80 weeks. Slight liver damage to a lesser degree than at 150 mg/kg diet. No kidney, lung, heart, adrenal, spleen or intestinal damage.	
				150		Slight reduction in growth rate after 60 weeks, greater in females, coinciding with periods of anorexia and tremors. Tremors after 26 weeks. Increased mortality at 48 weeks. Kidney and liver hypertrophy at 48 weeks in subgroup of 5 rats/sex. Upon histology duodenal ulcers and liver damage were revealed (centrilobular hypertrophy, nuclear karyorrhexis, karyolysis, necrosis, fatty degeneration, bile duct proliferation). Moderate to marked kidney, lung, myocardium, adrenal and spleen damage.	
				300		Reduced growth rate after 60 weeks, greater in females, coinciding with periods of anorexia and tremors. Hemorrhaging around eyes and nose associated with histological changes at 12 weeks. Increased mortality, 0 females and 1 male survived longer than 84 weeks. More severe liver, kidney, heart, adrenal, lung, myocardium and spleen damage.	
Dog/NR	NR/NR	NR	NR	0, 0.3, 3, 15 and 30	2 years	A review panel for WHO/FAO indicated that 3 mg/kg diet was a NOEL. The endpoints of the study were increased liver weight, and histopathologic changes in the liver.	Wazeter, 1967

*Unless otherwise stated

NR - Not reported

TWA - Time-weighted average

and two males died at the highest exposure level. Histological examinations were not performed or were not reported. Maximum tolerated doses were established at 400 and 800 mg/kg diet for female and male rats, respectively, and at 80 and 40 mg/kg diet for female and male mice, respectively.

Shain et al. (1977) found that feeding of technical grade chlordane to male Sprague-Dawley rats for 90 days at an average dose of 19.5 mg/kg bw/day had no effect on food consumption when compared with control-fed rats. Mean body weight gain was significantly reduced in a subgroup of 12 randomly selected rats. In another subgroup of 24 rats, body weight was not reduced and no changes were observed in testicular or ventral prostate weights. Histological examination of the rats was not performed, since the study was designed to determine the effect of chlordane on prostate homeostasis. Rats were castrated 24 hours before sacrifice. Significant changes from controls were as follows: increased nuclear (642%) but not cytoplasmic androgen receptor site content, decreased RNA content (35%) and decreased DNA content (25%). Ventral prostate protein content was also reduced to 13% of control.

Induction of hepatic microsomal enzymes during 13 weeks of feeding various levels of chlordane or transchlordane to rats was reported by Kinoshita and Kempf (1970) in a meeting abstract. Dose-related increases in activities of enzymes involved in phosphorothioate detoxication, O-demethylase and N-demethylase activation, maximal during the first week of treatment, were noted. The no-effect level for enzyme induction was 1 ppm (1 mg/kg diet).

DeLong and Ludwig (1954) found that administration of an average daily dose of 1.2 mg technical grade chlordane/kg bw in the diet of male and female rats for 5 months resulted in no histopathologic damage to lungs, heart, stomach, liver, kidney, spleen or testes. The rats were fed dog pellets that had been treated by fog application of spray concentrations of chlordane. The average daily dose was calculated by the investigators from food consumption data. There was no mention of how many animals were tested; however, the investigators stated that two control rats died. One treated rat had a kidney adenocarcinoma, which was believed to be unrelated to chlordane treatment.

Chronic Dietary Exposure. Table V-5 summarizes the studies discussed in this section.

Ambrose et al. (1953a,b) conducted a study on the dietary effects of technical grade chlordane fed to groups of male and female rats for 400 days. The dietary levels used were 0, 10, 20, 40, 80, 160, 320, 640 and 1280 ppm (mg/kg) diet. Increased mortality was observed in the 640 and 1280 ppm groups and retarded growth was observed in all animals at $\geq$320 ppm. Some growth retardation was observed in male rats fed 20, 40 or 160 ppm chlordane diets, but no growth retardation was observed in female rats fed $\leq$160 ppm or in the male rats fed 10 or 80 ppm. Significantly enlarged livers and liver pathology were found in male and female rats fed chlordane at $\geq$80 ppm and liver pathology was occasionally found in male rats fed 40 ppm. Necrotic or degenerative pathology were present in the study animals.

Ingle (1952) described the dose-response effects in male and female Osborne-Mendel rats that had been exposed to chlordane in the diet for 2 years. High mortality among males and females, reduced growth rates, eye and nose hemorrhaging and severe histopathologic damage to liver, kidney, heart, adrenal, lung, myocardium and spleen were observed at 300 ppm (300 mg/kg diet). At a 150 mg/kg diet level, similar but less severe effects were seen. The effects at the 30 mg/kg diet level included inducible tremors and slight liver damage. No effects on food consumption, growth rate, mortality, organ weights or morphology were observed at this dose level. A diet containing 10 mg chlordane/kg produced minor liver damage, such as occasional hepatocytomegaly and mild bile duct hyperplasia. There was no effect on food consumption, growth rate or mortality at the 5 mg/kg diet levels. No symptoms of toxicity, gross or histopathologic changes in liver, kidney, lungs, pancreas, testes, ovaries, heart or spleen were noted.

Several studies that were designed to assess the carcinogenic potential of chlordane following long-term dietary exposure of rats and mice provide information on nontumor pathology. Disagreement among pathologists in the diagnosis and distinction of hepatic carcinoma from pre-cancerous lesions and hyperplasia in rodents has confounded the assessment of increased incidence of hyperplasia as a toxic rather than a carcinogenic effect.

Becker and Sell (1979), in an attempt to resolve these differences, studied elevated alpha-fetoprotein levels as an indicator of chlordane carcinogenicity in male C57Bl/6N mice. The chlordane used was 90% chlordane and 10% heptachlor and was administered in the diet at concentrations of 0, 25 and 50 ppm (mg/kg diet) for at least 36 weeks to an unspecified number of male mice. At 25 and 50 mg/kg diet, 2 and 7%, respectively, of the mice had benign proliferative lesions defined as "clearly demonstrable foci, areas or nodular lesions that demonstrated neither microinvasion nor cytological atypia." No lesions were observed in 200 control mice. Elevated levels of alpha-fetoprotein were observed in treated mice after 38 weeks and was always associated with primary hepatocellular carcinoma. The investigators stated that of 12 mice that had benign lesions without carcinoma, none had elevated alpha-fetoprotein levels. No growth was detected 3-7 months after six of the benign lesions were transplanted to compatible hosts, while 5-12 transplanted carcinomas had shown growth after 1.5-3 months. Thus, the benign lesions did not appear to be premalignant lesions.

An unpublished report by the International Research and Development Corporation (IRDC, 1973a) under contract to the Velsicol Chemical Corporation was reviewed by Epstein (1976). Groups of 100 male and 100 female CD-1 mice were administered technical grade chlordane in the diet at concentrations of 0, 5, 25 or 50 ppm (mg/kg diet) for 18 months. An interim kill of 10 mice/sex/group was performed at 6 months. No effect on body weight gain or food consumption was observed. At both 6 and 18 months, mean liver weights of all treated female groups were significantly increased in a dose-related manner. At 6 months, the 50 mg/kg diet level group of males had significantly increased mean liver weights when compared with controls. Survival, although underestimated because of the interim kill, was 14% for males and 24% for females in the 25 mg/kg diet group and 51-73% for all other groups, including controls. Increased incidence of hepatocytomegaly in all treated female and male groups occurred in a dose-related manner.

The incidence of hyperplastic nodules, as reported by IRDC (1973a), was dose-related at the two highest dose levels ($p<1\times10^{-9}$). Re-evaluation of histologic slides by five of six other pathologists resulted in decreased incidences of the nodules in favor of greater incidences of carcinomas; however, total agreement was not obtained. Further discussion of this study is presented in the Carcinogenicity Section.

In the NCI (1977a) bioassay of the carcinogenicity of chlordane, groups of 50 male and 50 female Osborne-Mendel rats and groups of 50 of each sex $B6C3F_1$ mice were maintained on diets containing analytical grade chlordane (94.8%) for 80 weeks, followed by 29 and 10 weeks of observation for rats and mice, respectively. Controls for the rats consisted of 10 matched and 60 pooled untreated rats (controls from concurrent and recent bioassays of other related compounds) per sex. For male mice, 20 matched and 100 pooled controls were used, and for female mice, 20 matched and 80 pool controls were used. The doses were determined from the previously described preliminary subchronic study. Symptoms of toxicity and high mortality during the initial part of the study necessitated reductions in the dietary levels. A description of these adjustments, the dosing schedule and the resultant time-weighted average (TWA) dose, hereafter referred to as high doses and low doses, are presented in Tables V-6 and V-7 for rats and mice, respectively. For rats, mean body weight of high dose males and females were consistently depressed. High dose females had tremors at 44 weeks. All treated groups had individuals with symptoms of toxicity (loss of body weight, rough and discolored hair, palpable masses and tumors) that became progressively worse as the study continued. Among female rats there was a highly significant ($p=0.003$) dose-related increase in mortality. Mortality

TABLE V-6

Design Summary for NCI Bioassay of Chlordane in Osborne-Mendel Rats[a]

Sex and Treatment Group	Initial No. of Animals[b]	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
MALE					
Matched-Control	10	0	0	109	
Low-Dose	50	400	33		203.5
		100	14		
		50	33		
		0	0	29	
High-Dose	50	800	33		407.0
		200	14		
		100	33		
		0	0	29	
FEMALE					
Matched-Control	10	0	0	109	
Low-Dose	50	200	33		120.8
		100	14		
		50	33		
		0	0	29	

TABLE V-6 (cont.)

Sex and Treatment Group	Initial No. of Animals[b]	Chlordane in Diet (ppm)	Time on Study: Treated (weeks)	Time on Study: Untreated[c] (weeks)	Time-Weighted Average Doses[d] (ppm)
High-Dose	50	400	33		241.5
		200	14		
		100	33		
		0	0	29	

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[a] Source: NCI, 1977a

[b] All animals were 35 days of age when placed on test.

[c] When diets containing chlordane were discontinued, treated rats and their matched controls were fed plain feed diets (without corn oil) for 12 weeks and then control diets (2% corn oil added) for an additional 17 weeks.

[d] Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of days at that dose})}{\Sigma(\text{no. of days receiving each dose})}$

TABLE V-7

Design Summary for NCI Bioassay of Chlordane in B6C3F$_1$ Mice[a]

Sex and Treatment Group	Initial No. of Animals[b]	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
MALE					
Matched-Control	20[e]	0	0	91	
Low-Dose	50	20	1		29.9
		30	79		
		0	0	10	
High-Dose	50	40	15		56.2
		60	65		
		0	0	10	
FEMALE:					
Matched-Control	20[e]	0	0	91	
Low-Dose	50	40	1		30.1
		30	79		
		0	0	10	

TABLE V-7 (cont.)

Sex and Treatment Group	Initial No. of Animals[b]	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
High-Dose	50	80	15		63.8
		60	65		
		0	0	10	

[a] Source: NCI, 1977a

[b] All animals were 35 days of age when placed on test.

[c] When diets containing chlordane were discontinued, mice received the control diet (2% corn oil added) until termination.

[d] Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of days at that dose})}{\Sigma(\text{no. of days receiving each dose})}$

[e] Initially 10 animals of each sex were placed on test as matched controls; however, when the study was restarted, 10 additional animals of each sex were placed on test as matched controls.

was not significantly increased for male rats. Histopathologic signs of aging, such as chronic nephritis, biliary hyperplasia and chronic prostatitis, were observed with about equal frequency in all control and treated groups. The incidences of follicular and C-cell hyperplasia in thyroid glands of all treated and control groups were observed in a non-treatment-related incidence.

The results in mice indicated no difference in body weight gains among groups. Tremors were observed in high-dose males and females after 20 weeks. Mortality was significantly increased among treated males, but not females, in a dose-related manner. Histopathologic signs of aging commonly seen in mice occurred in all groups. Hepatocytomegaly and diffuse hepatic hyperplasia occurred infrequently in individuals of all groups.

In an unpublished study by Yonemura et al. (1983), F-344 rats (80/sex/group) were fed technical chlordane at dietary levels of 0, 1, 5 or 25 ppm for 130 weeks. Body weight, food consumption and water intake were monitored at regular intervals. Clinical laboratory studies were performed and organ weights were measured on eight animals/sex/group at weeks 26 and 52, and on all survivors at week 130. Gross and microscopic pathology were performed on all tissues. Daily dose levels of 0.045, 0.229 and 1.175 mg/kg for the 1, 5 and 25 ppm treatment groups, respectively, were calculated from food consumption and body weight data. No effects were observed for hematology, clinical chemistry and urinalysis endpoints, and no treatment related effects were reported for body weight and mortality. Hepatocellular necrosis was observed in 3, 13, 11 and 27 males (64/group) in the 0, 1, 5 and 25 ppm groups, respectively. The increased incidence was statistically significant for all treatment groups. Liver adenomas were found in the high-dose males. The only significant effect in females was hepatocellular swelling in the 25 ppm group.

Increased liver-to-body weight ratios were reported for male and female mice fed chlordane for 2 years at 0.76 ppm (0.09 mg/kg/day), the lowest dose administered (Inui et al., 1983). Liver necrosis was observed at 0.43 and 1.1 mg/kg/day for males only.

Four-week pilot studies in rats and mice were performed to establish the dose levels in these two studies. Both dietary studies resulted in liver lesions in the low-dose animals (50 ppm for rats and 10 ppm for mice).

An additional unpublished chronic study performed in dogs by Wazeter (1967) was reported by Vettorazzi (1975). The dogs were maintained for 2 years on diets containing chlordane at levels of 0, 0.3, 3, 15 or 30 ppm. The effects observed were increased liver weight and histologic changes in the liver. As reported by Vettorazzi (1975), a scientific review panel for WHO/FAO examined this study and concluded that no effects were observed at exposures of 3 mg/kg diet or less. Other specific data on the experimental design or results of this study were unavailable.

Other Routes of Exposure. No data were available on inhalation or dermal exposure; however, several investigators (Karel and Saxena, 1976; Singhal and Kacew, 1976; Hyde and Falkenberg, 1976) have described effects following intraperitoneal and intramuscular injection of chlordane. Details of the studies are summarized in Table V-8. Karel and Saxena (1976) found

TABLE V-8

Effects of Subchronic Exposure to Chlordane by Other Routes

Species/Strain	Sex/Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	References
Gerbil/Indian desert	M/64 group	70-80 g/adult	technical grade 60-75% pure	intramuscular	petroleum ether	0 and 2.5 mg/kg bw every 3 days	45 days	Increased blood glucose, serum protein, serum alkaline phosphatase and serum acid phosphatase levels of treated gerbils compared with controls.	Karel and Saxena, 1976
Rat/NR	NR/4/group	NR	cis-chlordane	intraperitoneal	corn oil	0 and 25 mg/kg bw/day	45 days	Significantly increased blood glucose, serum urea, decreased liver glycogen, increased kidney and liver pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase ($p<0.05$).	Singhal and Kacew, 1976
Rat/Sprague-Dawley	M/4/group	255-275 g	technical grade	intraperitoneal	Tween-80-isotonic saline	0, 0.15, 1.75, and 25.0 mg/kg bw/day	48 days	Dose-related changes in EEGs increasing as exposure increased without symptoms of toxicity	Hyde and Falkenberg, 1976

NR = Not reported

impaired liver function in gerbils, as evidenced by increased blood glucose, serum protein, acid and alkaline phosphatase, after intramuscular injection with 15 doses of chlordane (60-75% pure) 2.5 mg/kg bw over 45 days. Similar results were observed for increased activities of gluconeogenic enzymes in rats following intraperitoneal injections of cis-chlordane at 25 mg/kg bw/day for 45 days (Singhal and Kacew, 1976). Dose-related changes in EEG without symptoms of toxicity were observed in rats treated intraperitoneally with <25.0 mg/kg bw/day for 48 days (Hyde and Falkenberg, 1976).

Heptachlor and Heptachlor Epoxide --

Dietary Exposure. Table V-9 summarizes the studies discussed in this section.

A preliminary subchronic study was conducted to determine the dose levels of heptachlor to be administered to Osborne-Mendel rats and B6C3F$_1$ mice for carcinogenicity testing (NCI, 1977b). Male and female rats, five of each sex per group, were administered diets containing technical grade heptachlor (~73% heptachlor, 22% transchlordane, 5% nonachlor) in concentrations of 0-320 ppm (320 mg/kg diet) for 42 days. The rats were then maintained on heptachlor-free diets for 2 weeks. The only criteria for toxicity were food consumption, body weight gain and mortality. No effects on body weight gain or food consumption were observed at <40 mg/kg diet. At 80 mg/kg diet, female rats had reduced body weight during the first week. Diets containing 160 mg/kg diet resulted in deaths of four females. A dietary level of 320 mg/kg diet resulted in the deaths of two male and five female rats.

TABLE V-9

Effects in Rats and Mice of Subchronic and Chronic Dietary Exposure to Heptachlor and Heptachlor Epoxide

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet)[a]	Duration	Effects	Reference
Rat/Osborne-Mendel	M and F/ 5/sex/ group	NR	Technical heptachlor (73% heptachlor, 22% trans-chlordane, 5% nonachlor	0	42 days on test diet, 14 days observation		NCI, 1977b
				20		No effect on bw gain or food consumption compared with controls.	
				40		No effect on bw gain or food consumption compared with controls.	
				80		Females had decreased bw gain during first week.	
				160		4/5 females died, no effect on bw gain or food consumption of males.	
				320		2/5 males died, 5/5 females died.	
Mouse/B6C3F$_1$	M and F/ 5/sex/ group	NR	Technical heptachlor (73% heptachlor, 22% trans-chlordane, 5% nonachlor	0	42 days on test diet, 14 days observation		NCI, 1977b
				20		No effect on bw gain or food consumption.	
				40		No effect on bw gain or food consumption.	
				80		5/5 males, 2/5 females died.	
Rat/Sprague-Dawley	M/ 42/group	91 days	99.8% heptachlor	0 and 1.29 mg/kg bw (based on food consumption data)	90 days	Moderate but significant ($p<0.01$) reduction in food consumption compared with control rats. Decreased mean bw gain ($p<0.01$). No significant change in testicular or ventral prostate weights. Significantly increased cytoplasmic, but not nuclear, androgen receptor site content of ventral prostate ($p<0.05$). Decrease in available cytoplasmic androgen receptor site content ($0.05<p<0.01$). Decreased ventral prostate protein (13%) and cell loss (72%).	Shain et al., 1977
Rat/NR	M and F/ NR	NR	Heptachlor 98.1%	6 mg/kg bw[b]	52 weeks	Markedly decreased litter size, decreased lifespan of pups, development of cataracts.	Mestitzova, 1967

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet)[a]	Duration	Effects	Reference
Mouse/CD-1	M and F/ 100/sex/ group	7 weeks	Heptachlor:heptachlor epoxide (25:75)	0, 1.0, 5.0 and 10.0	18 months, interim kill at 6 months of 10 mice/sex/group	Females at 10 mg/kg diet had decreased, but not marked, bw gain compared with controls, no differences in food consumption. There was a dose-related increased mean liver weight in treated females and especially males at 6 and 18 months. These increases were significant for 5 and 10 mg/kg diet males and females at 18 months ($p<0.01$) and for 1 mg/kg females at 18 months ($p<0.05$). Survival was 29% for males and 30% for females at the 10 mg/kg diet level and 51-66% for all other groups. Neoplastic lesions of the liver were observed and are discussed in the Carcinogenicity Section. Hepatocytomegaly was observed in a dose-related increased incidence for all treated males and females. The incidence of hyperplastic nodules (described as an extension of the hepatocytomegaly) was dose-related at the 5 and 10 mg/kg diet levels for males and females and was significant ($p<1\times10^{-6}$). Re-evaluation of slides by other pathologists resulted in decreased incidences of the nodules in favor of greater incidences of carcinomas (see Carcinogenicity Section).	Epstein, 1976 (review of IRDC, 1973b)

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet)[a]	Duration	Effects	Reference
Rat/Osborne-Mendel	M/10 matched, 60 pooled controls	35 days	Technical heptachlor (~73% heptachlor, 22% trans-chlordane, 5% nonachlor	0	111 weeks	Consistently decreased bw in both high-dose males and females compared with controls; a greater effect in males. The bw of low-dose groups were similar to controls. Both treated and control groups developed adverse clinical symptoms. In some females of both treated groups, vaginal bleeding developed after 80 weeks on test diet. Some low-dose females had mammary tumors. A dose-related, however not significant (p=0.17) increase in mortality was observed in males. A dose-related significant increase in mortality (p=0.04) was observed between treated female groups. Histologic signs of aging occurred with equal frequency in control and treated rats. Neoplastic lesions were observed and are discussed in the Carcinogenicity Section. Hyperplastic lesions were observed in thyroid glands of rats of all groups. These were follicular- and C-cell hyperplasias.	NCI, 1977b
				TWA 38.9, 77.9	80 weeks on test diet, 21 weeks observation		
	F/10 matched, 60 pooled controls	35 days		0	111 weeks		
				TWA 25.7, 51.3	80 weeks on test diet, 21 weeks observation		
Mouse/B6C3F$_1$	M/20 matched, 100 pooled controls	35 days	Technical heptachlor (see above)	0	90-91 weeks	No appreciable differences in bw between treated and control mice were observed. Mice of all groups had sores and hair loss during first year. High dose females developed abdominal distention. Mice from all groups had rough coats, hair loss, sores and palpable masses. There was no apparent difference in male mortality among groups. There was a significant dose-related increase in mortality (p=0.02) between treated female groups. Hepatocytomegaly, diffuse hyperplasia and nodular hyperplasia were seen in livers of control and treated mice. Nonneoplastic lesions occurred in other organs randomly among all groups.	NCI, 1977b
				TWA 6.1, 13.8	80 weeks on test diet, 10 weeks observation		
	10/matched 80 pooled controls	35 days		0	90-91 weeks		
				TWA 9.0, 18.0	80 weeks on test diet, 10 weeks observation		

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet)[a]	Duration	Effects	Reference
Mouse/C3H	M/ 73 control 81 treated	3 weeks	Heptachlor purity NR	0 and 10	2 years	No hepatic vein or cardiac atrial thrombosis in controls. 13% of treated mice (10% of males and 15% of females) had venous occlusion with recent liver infarcts. Carcinomas and cirrhosis were also observed in some mice with thrombi. Of the mice with thrombi, 11% did not have carcinomas. A few mice with hepatic vein thrombosis had cardiac atrial thrombosis. Hyperplasia and hyperplastic nodules were observed in controls and treated mice.	Reuber, 1977a, 1978 (histologic slides were from study of Davis, 1965)
Mouse/C3H	M and F/ 100/group	3 weeks	Heptachlor purity NR	0 and 10	2 years	Survival was 34% for controls, 30% for treated mice. "Hepatic hyperplasia and benign tumors" were seen in 2- to 3-fold greater frequency in treated mice. Histologic re-evaluation of slides by other pathologists resulted in decreased incidences of hyperplasias and nodules in favor of increased incidence of carcinoma (see the Carcinogenicity Section).	Epstein, 1976 (review of Davis, 1965)
Rat/CF	M/20/group	10 weeks	Heptachlor purity NR	0 1.5 3.0 5.0 7.0 10.0	110 weeks	Mortality 40% 70% 65% 50% 40% 75% Loss of bw in treated males, most predominant in 10 mg/kg diet group. Increased liver weights in treated males especially in highest dose group. Reduced food consumption in 10 mg/kg diet group. Liver lesions described as "Chlorinated hydrocarbon" type seen in 30% of 7.0 mg/kg diet group, 17% in 10 mg/kg diet group and 0% in other groups.	Epstein, 1976 (review of Witherup et al., 1955)

TABLE V-9 (cont.)

Species/Strain	Sex/Number	Weight/Age	Compound and/or Purity	Dose (mg/kg diet)[a]	Duration	Effects		Reference
						Mortality		
Rat/CF (cont.)	F/20/group	10 weeks	Heptachlor purity NR	0 1.5 3.0 5.0 7.0 10.0	110 weeks	40% 40% 55% 35% 40% 55%	Loss of bw, increased liver weight, not seen in females. "Chlorinated hydrocarbon" type liver lesions seen in 17% of 7.0 mg/kg diet group. 50% of 10 mg/kg diet group and 0% in other groups. The incidence of malignant tumors is discussed in the Carcinogenicity Section.	
Rat/CD	F/ 54 control 25 treated/group	NR	99.9% heptachlor: 96%, heptachlor epoxide (75:25)	0 5 7.5 10.0 12.5	2 years	21% 39% 25% 43% 50%	Age related lesions seen in all groups. Spontaneous lesions and tumors seen in all groups (multiple cell type hypertrophy and telangiectasia in anterior pituitary and adrenal hypertrophy). Hepatocytomegaly exceeded control levels at 7.5, 10.0 and 12.5 mg/kg diet levels.	Epstein, 1976 (review of Jolley et al., 1966)
Mouse/C3H	M/ 79 treated F/ 81 treated	3 weeks	Heptachlor epoxide purity NR	0 and 10 ppm	2 years		No hepatic vein or cardiac atrium thrombi, no cirrhosis of liver in controls. 10% of treated mice (7% of males, 11% of females) developed hepatic vein thrombosis; 9% (5 males, 9 females) treated mice had venous occlusion. Carcinomas and cirrhosis were observed in some mice with thrombi. Of the mice with thrombi, 11% did not have carcinomas. A few had cardiac atrium thrombosis as well. 12/78 treated males, 15/81 treated females had cirrhosis.	Reuber, 1977a, 1978 (histologic slides obtained from study of Davis, 1965)

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TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet)[a]	Duration	Effects		Reference
Mouse/C3H	M and F/ 100/group	NR	Heptachlor epoxide purity NR	0 and 10	2 years	Survival was 34% for controls, 9.5% for treated mice. Premature death was high. "Hepatic hyperplasia and benign tumors" were seen in 2 fold greater frequency in treated mice. Histologic re-evaluation of the slides by other pathologists resulted in decreased incidences of hyperplasias and nodules in favor of increased incidences of carcinomas (see Carcinogenicity Section).		Epstein, 1976 (review of Davis, 1965)
Rat/CFN	M/25/group	7 weeks	Heptachlor epoxide purity NR	0 0.5 2.5 5.0 7.5 10.0	110 weeks	Mortality 32% 48% 52% 48% 40% 44%	No difference in food consumption or growth rate. Hepatic cell vacuolization in all treated groups. Hepatocytomegaly, degeneration and regeneration in unspecified groups. Tumor data are discussed in the Carcinogenicity Section.	Epstein, 1976 (review of Witherup et al., 1959)
	F/25/group	7 weeks		0 0.5 2.5 5.0 7.5 10.0	110 weeks	24% 44% 36% 28% 52% 48%	No food consumption differences, no differences in growth rate. Dose-related increase in liver weights. Hepatic cell vacuolization in all treated groups. Hepatocytomegaly, degeneration and regeneration in unspecified groups. Tumor data are discussed in the Carcinogenicity Section.	

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[a] mg/kg diet unless otherwise stated

[b] It is unclear from the study whether or not this is a daily dose and administered by feeding. The author stated that heptachlor was "administered with food" and "the applied dose was 6 mg/kg bw".

NR - Not reported; TWA - Time weighted average

Groups of five male and five female mice received diets containing 0-80 ppm (0-80 mg/kg diet) heptachlor for 42 days followed by 14 days observation (NCI, 1977b). No deaths and no effects on body weight gain or food consumption occurred at <40 mg/kg diet. At 80 mg/kg diet, all male and two female mice died. Histological examinations of the rats or the mice were not performed or were not reported. Maximum tolerated doses were established at 160 and 80 mg/kg diet for male and female rats, respectively, and 40 mg/kg diet for male and female mice.

Shain et al. (1977) found that feeding of heptachlor (99.8%) to male Sprague-Dawley rats for 90 days at an average dose of 1.29 mg/kg bw/day significantly reduced the food consumption and mean body weight gain in a subgroup of 12 randomly selected rats. In another subgroup of 22 rats, body weight was not reduced and no changes were observed in testicular or ventral prostate. Histological examination of the rats were not performed, since the study was designed to determine the effect of heptachlor on prostate homeostasis. Rats were castrated 24 hours before sacrifice. Cytoplasmic, but not nuclear, androgen receptor site content of the ventral prostate was significantly increased. Ventral prostate protein content was reduced to 13% of control levels and cell number was reduced to 18% of control.

Induction of hepatic microsomal enzymes during 13 weeks of feeding various dietary levels of heptachlor or heptachlor epoxide to rats was reported by Kinoshita and Kempf (1970) in a meeting abstract. There were dose-related increases in the activities of enzymes involved in phosphorothioate detoxication and activities of O-demethylase and N-demethylase, maximal during the first week of treatment. The no-effect level for enzyme induction was reported to be 1 ppm (1 mg/kg diet).

Mestitzova (1967) found that heptachlor (98.1%) administered with food at a dose of 6 mg/kg bw for 52 weeks resulted in markedly decreased litter sizes, decreased lifespan of pups and the development of cataracts in parent rats and pups. It is unclear whether the 6 mg/kg bw dose was administered daily or whether it represented a total dose consumed over 1 year. In the former case, the dose would be quite high, roughly corresponding to a concentration of 120 mg/kg diet, assuming that a rat consumes 5% daily of its body weight as food. In the latter case, the dose would be quite low, resulting in a daily average of ~0.016 mg/kg bw/day.

As with chlordane, several long-term feeding studies with heptachlor or heptachlor epoxide were designed as carcinogenicity studies but provide toxicologic data as well, and there has been disagreement among pathologists on the distinction between malignant and benign lesions of the liver induced by heptachlor or its epoxide.

In the unpublished report by IRDC (1973b) reviewed by Epstein (1976), a 25:75 mixture of heptachlor:heptachlor epoxide was administered to groups of 100 male and 100 female CD-1 mice in the diet at 0, 1.0, 5.0 and 10.0 ppm (mg/kg diet) for 18 months. An interim kill of 10 mice/sex/group was performed at 6 months. Female mice at the 10 mg/kg diet level had decreased body weight gain; otherwise, no other effect on body weight or food consumption was observed. Mean liver weight increased significantly in a dose-related manner in treated females and males at 6 and 18 months. This increase was more marked in males. Survival, although underestimated due to the interim kill, was 29% for males and 30% for females at the highest dose level and 51-66% for all other groups, including controls. Increased incidence of hepatocytomegaly in all treated female and male groups occurred in a dose-related manner. The incidence of hyperplastic nodules, as reported by IRDC (1973a), was dose-related at the two highest levels for males and females and was highly significant ($p<1x10^{-9}$). Re-evaluation of histologic slides by six other pathologists resulted in several different interpretations; however, the overall result was a decreased incidence of hyperplasia in favor of a greater incidence of carcinoma. Further discussion of this study is presented in the Carcinogenicity Section.

Epstein (1976) reviewed the results of several other unpublished reports: 1) Davis (1965), an FDA report on heptachlor and heptachlor epoxide; 2) Witherup et al. (1955), a study by the Kettering Laboratories on heptachlor; 3) Jolley et al. (1966), a study by Kettering on a heptachlor and heptachlor epoxide mixture; and 4) Witherup et al. (1959), a Kettering study on heptachlor epoxide. Discussion of these studies with respect to toxic effects follows. Further discussion of the carcinogenic effects is presented in the Carcinogenicity Section.

Davis (1965) administered 0 and 10 ppm (10 mg/kg diet) heptachlor or heptachlor epoxide to groups of 100 male and 100 female C3H mice for 2 years. For heptachlor, low survival was observed for treated (30%) as well as control (34%) mice. For heptachlor epoxide, survival was 9.5%. A 2-fold increase over controls in the incidence of hepatic hyperplasia and benign tumors was observed for both compounds, although re-evaluation of the slides by four other pathologists changed the interpretation in favor of a greater number of hepatomas.

Witherup et al. (1955) studied the effects of heptachlor on groups of 20 male and 20 female CF rats. The insecticide was administered in dietary concentrations of 0-10 ppm (10 mg/kg diet). Mortality among test groups was not dose-related (see Table V-9). Loss of body weight, decreased food consumption and increased liver weight were seen among treated males, but not females, and were most marked at 10 mg/kg diet. Liver lesions described as "chlorinated hydrocarbon" type and considered to be non-neoplastic were observed in 17% of males and 50% of females at 10 mg/kg diet, and 38% of males and 17% of females at 7 mg/kg diet. At dose levels at or below 5.0 mg/kg diet, none of these lesions was observed. This study was not reevaluated by other pathologists.

Jolley et al. (1966) administered a 75:25 mixture of heptachlor: heptachlor epoxide to groups of 25 female CD rats in the diet at concentrations 5-12.5 ppm (12.5 mg/kg diet) for 2 years. A group of 54 rats received diets free of insecticide. Mortality was increased in a somewhat dose-related way (see Table V-9). Comprehensive histological evaluations were performed. Spontaneous lesions and tumors were present in all groups and included multiple cell type hypertrophy, telangiectasia in the anterior pituitary and adrenal hypertrophy. The incidence of hepatocytomegaly was increased over control levels at 7.5, 10.0 and 12.5 mg/kg diet. This study was not reevaluated by other pathologists.

Witherup et al. (1959) studied the effect of heptachlor epoxide in groups of 25 male and 25 female CFN rats for 110 weeks. The dietary concentrations ranged from 0.5-10.0 ppm (mg/kg diet). Control rats received heptachlor epoxide-free diets. No differences were observed with respect to food consumption or growth rate. There was a dose-related increase in liver weight in females. Hepatic cell vacuolization occurred in treated males. Hepatocytomegaly, degeneration and regeneration in unspecified groups were reported. Mortality was higher than control level for all treated groups, but a dose-response relationship was not observed. This study was not re-evaluated by other pathologists.

Reuber (1977a, 1978), using slides from the study of Davis (1965), found hepatic vein thrombosis and cirrhosis among the heptachlor and heptachlor epoxide (10 mg/kg diet) treated mice. These conditions were not observed in any of the 127 control slides available for review. For heptachlor-treated mice, 13% (10% of males, 15% of females) had hepatic vein thrombosis and 6% had venous occlusion with recent liver infarcts. Thrombosis of the cardiac atrium was also present in some mice with hepatic vein thrombosis. The incidence of cirrhosis was 2/86 treated males and 5/77 treated females. For heptachlor epoxide, 10% of treated mice (7% of males, 11% of females) had hepatic vein thrombosis and 9% had venous occlusion. Cardiac atrium thrombosis was present in some mice. The incidence of cirrhosis was 12/78 treated males and 15/81 treated females. In addition, liver carcinomas were also observed in the treated mice.

In the NCI (1977b) bioassay of the carcinogenicity of heptachlor, groups of 50 male and 50 female Osborne-Mendel rats and groups of 50 of each sex B6C3F1 mice were maintained on diets containing technical grade heptachlor for 80 weeks plus 21 and 10 weeks of observation for rats and mice, respectively. Controls for the rats consisted of 10 matched and 60 pooled untreated rats (controls from concurrent and recent bioassays of other related compounds) per sex. For male mice, 20 matched and 100 pooled controls were used, and for female mice, 10 matched and 80 pooled controls were used. The doses were determined from the previously described preliminary subchronic study. Changes in dose levels were necessitated by developing symptoms of toxicity. A description of these adjustments, the dosing schedule and the resultant TWA doses, hereafter referred to as high doses and low doses, are presented in Tables V-10 and V-11 for rats and mice, respectively.

For rats, mean body weight of high-dose males and females were consistently depressed especially in males. The low-dose groups had growth rates similar to controls. Adverse clinical signs such as loss of body weight, rough and discolored hair and palpable masses, developed in treated and untreated groups. In some females from both dose groups, vaginal bleeding developed after 80 weeks. A dose-related, but not significant, increase in mortality was observed for treated males. The increase was significant between treated female groups. Histopathologic signs of aging, such as chronic nephritis, biliary hyperplasia and chronic prostatitis, were observed with about equal frequency in all control and treated groups. The incidence of follicular- and C-cell hyperplasia in thyroid glands of treated and control groups were observed to be nontreatment-related.

The results in mice indicated no differences in body weight gains among groups. Sores and hair loss occurred in treated and control mice during the first year. Abdominal distention and hair loss was prevalent in high-dose females. Adverse clinical signs developed in mice of all groups. Male mortality was unaffected; however, there was a significant dose-related increase in mortality between treated female groups. Hepatocytomegaly and diffuse hyperplasia occurred infrequently in individuals of all groups.

TABLE V-10

Design Summary for NCI Bioassay of Heptachlor in Osborne-Mendel Rats[a]

Sex and Treatment Group	Initial No. of Animals[b]	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
MALE					
Matched-Control	10	0	0	111	
Low-Dose	50	80	31		
		20	14		38.9,
		10	35		
		0	0	30	
High-Dose	50	160	31		
		40	14		77.9
		20	35		
		0	0	30	
FEMALE					
Matched-Control	10	0	0	111	
Low-Dose	50	40	22		
		60	9		25.7
		20	14		
		10	35		
		0	0	30	

TABLE V-10 (cont.)

Sex and Treatment Group	Initial No. of Animals[b]	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
High-dose		80	22		51.3
		120	9		
		40	14		
		20	35		
		0	0	30	

[a] Source: NCI, 1977b

[b] All animals were 35 days of age when placed on test.

[c] When diets containing heptachlor were discontinued, treated male rats and their matched controls were fed plain feed diets (without corn oil) for 11 weeks, then control diets (2% corn oil added) for an additional 18 weeks; treated female rats and their matched controls were fed plain feed diets for 9.5 weeks, then control diets for an additional 20 weeks.

[d] Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of days at that dose})}{\Sigma(\text{no. of days receiving each dose})}$

TABLE V-11

Design Summary for NCI Bioassay of Heptachlor in B6C3F$_1$ Mice[a]

Sex and Treatment Group	Initial No. of Animals[b]	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
MALE					
Matched Control	20[e]	0	0	90-91	
Low-Dose	50	10 5 0	18 62 0	10	6.1,
High-Dose	50	20 10 0	17 50 0	10	13.8
FEMALE					
Matched-Control	10	0	0	90-91	
Low-Dose	50	20 10 5 0	17 13 50 0	10	9.0

TABLE V-11 (cont.)

Sex and Treatment Group	Initial No. of Animals[b]	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses[d] (ppm)
			Treated (weeks)	Untreated[c] (weeks)	
High-Dose	50	40	17		18.0
		20	13		
		10	50		
		0	0	10	

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[a] Source: NCI, 1977b

[b] All animals were 35 days of age when placed on test.

[c] When diets containing heptachlor were discontinued, mice received control diet (2% corn oil added) until termination.

[d] Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm x no. of days at that dose})}{\Sigma(\text{no. of days receiving each dose})}$

[e] Initially 10 males were placed on test as matched controls.

Other Routes of Exposure. No data were available on inhalation or dermal exposure; however, several investigators (Kacew et al., 1973; Singhal and Kacew, 1976; Hrdina et al., 1974) have described effects following intraperitoneal and intramuscular injections of heptachlor and heptachlor epoxide. Details of the studies are summarized in Table V-12. Kacew et al. (1973) administered heptachlor or heptachlor epoxide to Wistar rats intramuscularly for 45 days. The doses were 0, 3 or 15 mg/kg bw/day for heptachlor and 0, 1 or 5 mg/kg bw/day for heptachlor epoxide. For both compounds, liver weights were decreased in all treated rats when compared with controls. Dose-related increases in enzymes of gluconeogenic metabolism in livers and kidneys were significant. Similar results were obtained following intraperitoneal injections of heptachlor (Singhal and Kacew, 1976). Hrdina et al. (1974) administered 0, 3 or 15 mg/kg bw/day heptachlor or heptachlor epoxide by an unspecified route to rats for 45 days and observed significantly decreased cerebro-cortical acetylcholine levels.

Target Organ Toxicity

Chlordane.

Effects on the Liver -- Histopathologic changes in the liver were observed in rats at dietary levels of chlordane >18 mg/kg diet for males and females (Ambrose et al., 1953a,b). At exposures as low as 10 mg/kg diet, female rats had slight increases in liver weight. Increased incidences of hepatocytomegaly, hepatic hyperplasia and nodules were reported in a carcinogenicity study (IRDC, 1973a); however, re-examination of slides indicated that the nodules were hepatomas (Epstein, 1976). Chlordane feeding induced benign proliferative lesions in the liver of a strain of mice that historically and concurrently did not develop lesions when untreated (Becker

TABLE V-12

Effects of Subchronic Exposure to Heptachlor and Heptachlor Epoxide by Other Routes

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose (mg/kg bw day)	Duration	Effects	Reference
Rat/ Wistar	M/4/ group	100 g	heptachlor purity NR	i.m.	corn oil	0, 3 or 15	45 days	No significant effect on bw, kidney, adrenal, thymus or heart weights at either dose level. Significantly decreased liver weights at both dose levels ($p<0.05$) and significantly decreased testes weight at 15 mg/kg bw/day ($p<0.05$) when compared with controls. Dose-related levels significantly increased over control levels of kidney and liver pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase ($p<0.05$). Dose-related, significantly elevated levels of blood glucose and serum urea and decreased liver glycogen ($p<0.05$).	Kacew et al., 1973
Rat/NR	NR/ 4/group	NR	heptachlor purity NR	i.p.	corn oil	0 and 15	45 days	Significantly increased blood glucose, serum urea, liver and kidney levels of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase; significantly decreased liver glycogen compared with controls ($p<0.05$).	Singhal and Kacew, 1976
Rat/NR	NR/NR	NR	heptachlor purity NR	NR	NR	0, 3 and 15	45 days	Significantly decreased cerebro-cortical acetylcholine; no change in brain-stem norepinephrine; significantly decreased brain stem 5-hydroxytryptophan.	Hrdina et al., 1974

TABLE V-12 (cont.)

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose (mg/kg bw day)	Duration	Effects	Reference
Rat/ Wistar	M/4/ group	100 g	heptachlor epoxide purity NR	i.m.	corn oil	0, 1 or 5	45 days	No significant effect on bw, kidney, adrenal, thymus testes or heart weights at either dose level. Significantly decreased liver weights at both dose levels. Dose-related levels significantly increased over control levels of liver and kidney pyruvate carboxylase, phosphenopyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase ($p<0.05$). Dose-related, significantly elevated levels of blood glucose and serum urea; decreased liver glycogen ($p<0.05$).	Kacew et al., 1973
Rat/NR	NR/NR	NR	heptachlor epoxide purity NR	NR	NR	0, 1 or 5	45 days	Significantly decreased cerebro-cortical acetylcholine level, no change in brain stem norepinephrine levels.	Hrdina et al., 1974

NR= Not reported

i.m.= intramuscular

i.p.= intraperitoneal

and Sell, 1979). The incidence of hepatocytomegaly and hyperplasia was not increased over control incidence in B6C3F1 mice (NCI, 1977a). Dose-related, increasingly severe liver damage was observed by Ingle (1952) in rats treated chronically with dietary levels of chlordane. The damage included centrilobular hypertrophy, nuclear karyorrhexis, karyolysis, necrosis, fatty degeneration and bile duct proliferation. Reticulation and oxyphilia of liver cytoplasm was observed after acute inhalation exposure (Ingle, 1953).

Several investigators noted changes in liver function, as evidenced by enzyme activity changes. Kinoshita and Kempf (1970) found that the activities of the microsomal enzymes, such as phosphorothioate detoxicating enzyme, O-demethylase and N-demethylase, were significantly increased in rats following technical grade chlordane or transchlordane feeding. Induction of liver aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase in rats was observed by Den Tonkelaar and Van Esch (1974). Enhancement of gluconeogenesis was described in rats, as evidenced by increased blood glucose, increased serum urea, decreased liver glycogen, increased activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase (Singhal and Kacew, 1976; Kacew and Singhal, 1973).

Effects on Endrocrine System and Reproductive Organs -- Hyperplastic lesions, such as follicular-cell and C-cell hyperplasia of the thyroid, were observed in rats treated with 120-407 mg/kg bw of chlordane (NCI, 1977a). Shain et al. (1977) found changes in ventral prostate homeostasis of rats at dose levels of ~20 mg/kg bw, as evidenced by increased nuclear androgen receptor site content and decreased protein, RNA and DNA content. Delayed vaginal opening, increased ovary weight and decreased pituitary weights were seen in neonatal mice treated with 0.075 mg/mouse (Talamantes and Jang, 1977).

Effects on CNS -- Clinical and behavioral manifestations of chlordane poisoning have been noted in rats and mice. Tremors, hyperexcitability, irritability, phonation, piloerection and convulsions were commonly observed after acute exposures (Lehman, 1951; Gaines, 1960; Boyd and Taylor, 1969; Stohlman et al., 1950; Arnold et al., 1977; Hrdina et al., 1974; Hyde and Falkenberg, 1976) and chronic exposures (Ingle, 1952; NCI, 1977a). Hyde and Falkenberg found dose-related (between 0.15 and 25 mg/kg bw/day) changes in EEGs of rats given daily intraperitoneal injections of chlordane.

Effects on Other Organs -- Moderate to marked histopathologic damage to kidneys, lungs, myocardia, adrenals and spleen in rats fed chlordane at 150 mg/kg diet became more severe at 300 mg/kg diet (Ingle, 1952). Duodenal ulcers and hemorrhaging around eyes and nose were also observed. Congestion and proliferation of bronchiole lining cells were seen in lungs of mice after acute inhalation exposure to chlordane (Ingle, 1953). Increased activities of kidney cortical pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase were observed in rats after acute oral exposure to chlordane (Kacew and Singhal, 1973).

Heptachlor and Heptachlor Epoxide.

Effects on the Liver -- Moderate to severe histopathologic changes in the liver were accompanied by elevated serum GPT and serum aldolase levels in rats after acute or subchronic oral exposures to heptachlor up to 14 days. At 28 days, however, the serum enzyme levels returned to control levels, and liver damage was less severe (Krampl, 1971). Similar findings by Enan et al. (1982) also included elevated serum bilirubin and serum cholesterol, decreased hepatic lipid content, decreased liver glycogen, increased serum glutamate-oxalacetate transaminase, increased alkaline phosphatase and decreased acid phosphatase levels after oral exposure of rats to heptachlor. Increased incidences of hepatocytomegaly, diffuse hepatic hyperplasia and hyperplastic nodules were common findings in mouse livers after chronic feeding of heptachlor and heptachlor epoxide (IRDC, 1973b; NCI, 1977b; Davis, 1965; Witherup et al., 1955, 1959; Jolley et al., 1966); however, re-evaluation of slides from the IRDC (1973b) study and of the Davis (1965) study, indicated that the hyperplastic conditions had been overdiagnosed by the original investigators and that the conditions of hepatocellular carcinoma had been underdiagnosed (Epstein, 1976). Cirrhosis and hepatic vein thrombosis were observed by Reuber (1977a) in mice treated with heptachlor or heptachlor epoxide.

Several investigators noted changes in liver function, evidenced by changes in enzyme activities. The microsomal enzymes of phosphorothioate detoxication, O-demethylase and N-demethylase activities increased in rat livers after dietary exposure to heptachlor or heptachlor epoxide (Kinoshita and Kempf, 1970). Induction of liver aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase occurred in rats after oral exposure to heptachlor (Den Tonkelaar and Van Esch, 1974). Decreased liver weights were accompanied by enhanced gluconeogenesis in rats following intramuscular or intraperitoneal injections of heptachlor or heptachlor epoxide (Kacew et al., 1973; Singhal and Kacew, 1976). Increased liver pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase activities; increased blood glucose and serum urea; and decreased liver glycogen were evidence of enhanced gluconeogenesis.

Effects on Endocrine System and Reproductive Organs -- Increased but not significantly dose-related incidence of hyperplastic lesions of the thyroid, including follicular-cell and C-cell hyperplasia, were observed in rats following chronic dietary heptachlor exposure (NCI, 1977b). Increased cytoplasmic nuclear androgen receptor site content of the ventral prostate, decreased prostate protein content and cell loss were indicative of changes in ventral prostate homeostasis in rats exposed to heptachlor (Shain et al., 1977). Kacew et al. (1973) observed decreased testes weights in rats following intramuscular injections of heptachlor. Testes weight was not increased following heptachlor epoxide injection. Vaginal bleeding developed in female rats after chronic dietary exposure to heptachlor (NCI, 1977b); however, the incidence was low and not significantly different from controls.

Effects on CNS -- Clinical and behavioral manifestations of heptachlor and heptachlor epoxide poisoning were observed in rats and mice. Tremors, hyperexcitability, irritability, convulsions, hind leg paralysis, hypoactivity and hypothermia were common findings after acute exposures to heptachlor and heptachlor epoxide (Lehman, 1951; Gaines, 1960; Hrdina et al., 1974; Arnold et al., 1977; Yamaguchi et al., 1980). Hrdina et al. (1974) found decreased levels of cerebrocortical acetylcholine and brain stem 5-hydroxy-tryptophan associated with tremors and convulsions.

Effects on Other Organs -- In addition to the hepatic vein thrombosis observed in mice following heptachlor or heptachlor epoxide feeding, a few cases of thrombosis of cardiac atrium were observed (Reuber, 1977a). In the kidney cortex, enzymes associated with gluconeogenesis have increased activities in rats after exposures to heptachlor or heptachlor epoxide (Kacew et al., 1973). Mestitzova (1967) reported that rats fed heptachlor developed cataracts of the eye.

Other Effects

Studies on the carcinogenicity, mutagenicity, teratogenicity and reproductive effects of chlordane, heptachlor and heptachlor epoxide exposure in laboratory animals are discussed in this section.

Carcinogenicity.

Animal Studies-Chlordane -- Chlordane has been studied in four mouse and four rat long-term carcinogenesis bioassays. Tables V-13 and V-14 present a summary of the experimental design and tumor results. Each study is described in more detail in the following sections.

TABLE V-13

Summary of Mouse Dietary Carcinogenicity Tests for Chlordane

Strain	No./Sex	Dose (ppm)	Duration of Exposure/ Observation	Tumor Results	Reference
C57Bl/6N	NS	25 or 50	18 months	Hepatocellular carcinomas in 27% (16) of the survivors. No other information on tumor incidences and time of death was presented.	Becker and Sell, 1979
CD-1	100/M&F	5, 25 or 50	18 months	Liver nodules at 25 and 50 ppm (IRDC); hepatocellular carcinomas at 25 and 50 ppm (Reuber)	IRDC, 1973a; Epstein, 1976
B6C3F1	50/M&F	29.9 and 56.2, M; 30.1 and 63.8, F	80 weeks plus 10 weeks observation	Hepatocellular carcinomas in both males and females	NCI, 1977a
ICR	80/M&F	1, 5 or 12.5	24 months	Hepatocellular adenomas and hemangiomas in 12.5 ppm males; nontumor liver lesions in females at 5 and 12.5 ppm and males at 5 ppm	RIASBT, 1983a

NS = Not specified

TABLE V-14

Summary of Rat Dietary Carcinogenicity Tests for Chlordane

Strain	No./Sex	Dose (ppm)	Duration of Exposure/ Observation	Tumor Results	Reference
Albino	3-5/M&F	10, 20, 40, 80, 160, 320 640, 1280	400 days	No treatment-related tumors; liver lesions at 80 ppm and above	Ambrose et al., 1953a,b
Osborne-Mendel	20/M&F	5, 10, 30, 150 or 300	2 years	High toxicity at 150 and 300 ppm; no treatment-related tumors	Ingle, 1952
Osborne-Mendel	50/M&F	203.5 and 407 ppm, M; 120.8 and 241.5 ppm, F	80 weeks plus 29 weeks observation	Toxicity in all groups; high mortality in females. Neoplastic nodules of liver in low-dose females; possible thyroid tumors in both males and females	NCI, 1977a
Fischer 344	80/M&F	1, 5 or 25	130 weeks	Hepatocellular adenomas in high-dose males; nonneoplastic lesions of liver at all doses in males and at high dose in females	RIASBT, 1983b

Studies with Mice.

Becker and Sell (1979) -- A 90:10 mixture of chlordane/heptachlor was fed to an unspecified number of male C57Bl/6N mice at concentrations of 25 or 50 ppm (~3.57 or 7.14 mg/kg bw) for 18 months. Specific information as to treatment and observation periods and time of death was not provided. The C57Bl/6N mouse rarely develops spontaneous liver lesions and in a group of 200 control mice no liver tumors or nodular lesions were found over 18 months of observation. In mice receiving the chlordane/heptachlor diet, many liver lesions were seen, including both benign proliferative lesions and hepatocellular carcinomas. Of those surviving to the end of the experiment, 27% (16 mice) had primary hepatocellular carcinomas, with the first appearing at 36 weeks. No other information was presented as to early deaths and associated tumor incidences. An even greater number of mice had benign proliferative lesions. Data relating tumor incidence to dose were not available. Elevated levels of alpha-fetoprotein were associated with animals with primary hepatocellular carcinomas. Cells from the carcinomas grew when transplanted, producing tumors that were histologically similar to the primary hepatocellular carcinoma. Cells from the benign lesions did not grow when transplanted. The authors concluded that the benign lesions did not appear to be premalignant lesions.

In a companion study, acetylaminofluorene was administered to the same strain of mice (C57Bl/6N) at 0.045 or 0.03% diet. After 61 weeks, seven (18%) of the survivors had liver carcinomas, and three showed growth at 2.4-4 months when transplanted. On a comparison basis, chlordane was considered to be a more potent carcinogen than acetylaminofluorene. Another key finding in this study was that chlordane induced hepatocellular carcinomas in a strain of mice that does not spontaneously develop hepatocellular carcinomas.

The International Research and Development Corporation (IRDC, 1973a) -- IRDC, under contract to the Velsicol Chemical Corporation, administered analytical grade chlordane in the diet at concentrations of 0, 5, 25 or 50 ppm (~0, 0.71, 3.57 or 7.14 mg/kg bw) for 18 months to groups of 100 male and 100 female CD-1 mice. The mice were 6 weeks of age when exposure began. A 6-month interim sacrifice of 10 mice/sex/group did not reveal compound-related lesions. No effect of chlordane on body weight gain or food consumption was observed. However, the survival was greatly reduced at the high-dose levels. A large number of animals were also lost due to autolysis. Only about one-half of the mice were histologically examined. A dose-related increased incidence of hepatocytomegaly was seen in all treated female and male groups. The incidence of hyperplastic liver nodules, as reported by IRDC (1973a), was dose-related at the two highest dose levels ($p<0.01$). For males, the incidences were: 1/47 (2%), 34/52 (65%) and 38/50 (76%) at 0, 25 and 50 ppm, respectively; whereas for females, the incidences were 0/57 (0%), 32/51 (63%) and 36/51 (71%), respectively. The number of hepatomas was higher, although not statistically at $p=0.05$, in the 25 ppm group (12) vs. that in the control (5) or 5 ppm groups (6). Only four hepatomas were diagnosed at 50 ppm; however, early deaths and a large amount of autolysis markedly reduced the number of mice at risk. Dr. Reuber reexamined the IRDC slides and found highly significant ($p<1x10^{-[illegible]}$) incidences of hepatic carcinoma rather than hyperplastic nodules at the 25 and

50 mg/kg diet levels. Three other independent pathologists (Drs. R. Squire, H. Stewart and H. Popper) examined subsets of the same slides examined by Reuber and were in close agreement with the diagnosis. Table V-15 presents a breakdown of liver lesions as diagnosed by Reuber. The incidences of hepatic carcinoma, as determined by Reuber, are presented in Table V-16, along with those found in the NCI study to be discussed next.

National Cancer Institute (1977a) -- In the NCI bioassay, groups of 50 male and 50 female B6C3F1 mice were fed chlordane consisting of 71.7% cis-chlordane, 23.1% trans-chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene, and 0.25% chlordene isomers in the diet for 80 weeks, at two doses -- a predicted maximum tolerated dose (MTD) and 1/2 MTD. This was followed by a 10-week observation period. As upward or downward adjustments were made in dose levels, the doses are expressed as time-weighted average (TWA) concentrations. The TWA concentrations for male mice at the high and low doses were 29.9 and 56.2 ppm (~4.27 and 8.03 mg/kg bw), respectively, and 30.1 and 63.8 ppm (~4.3 and 9.11 mg/kg bw), respectively, for female mice. Controls consisted of 20 matched control mice of each sex and 100 and 80 pooled male and female control mice, respectively. The results revealed no differences in body weight gain among groups. Tremors were observed in high-dose males and females after 20 weeks. A dose-related increase in mortality was seen in treated males, but not in females. A statistically higher ($p<0.001$) incidence of hepatocellular carcinoma was found in both males and females (see Table V-16).

TABLE V-15

Incidence of Liver Lesions and Tumors in CD-1 Mice Following Dietary Administration of Chlordane[a]

Dose (ppm)	Sex	No. of Mice Examined	No. of Mice Exhibiting Liver Lesions and Tumors[b]				
			H	N	SC	LC	TC
0	M	33	20 (61)	1 (3)	0	3 (9)	3 (9)
	F	45	26 (58)	0	0	0	0
5	M	55	34 (62)	6 (11)	3 (5)	2 (4)	5 (9)
	F[c]	61	32 (52)	1 (2)	0	0	0
25	M	52	7 (13)	3 (6)	9 (17)	32 (62)	41 (79)
	F	50	13 (26)	4 (8)	7 (14)	25 (50)	32 (64)
50	M	39	7 (18)	0	4 (10)	28 (72)	32 (82)
	F	37	11 (30)	0	2 (5)	24 (65)	26 (70)

[a] Source: IRDC, 1973a, breakdown of liver lesions diagnosed by Reuber (Epstein, 1976)

[b] H = hyperplasia; N = nodules; SC = small carcinomas (including hyperplastic nodules with focal carcinomas); LC = large carcinomas $\geq$5 mm; TC = total carcinomas

[c] Two liver sarcomas diagnosed in the 5 ppm female group

TABLE V-16

Incidence of Hepatocellular Carcinoma in Mice Following Chronic Dietary Administration of Chlordane

Dose (ppm)	No. of Tumor-Bearing Mice/No. of Mice Examined (% positive)	
	Male	Female
	Strain CD-1[a]	
0	3/33 (9%)	0/45 (0%)
5	5/55 (9%)	0/61 (0%)
25	41/52 (79%)	32/50 (64%)
50	32/39 (82%)	26/37 (70%)
	Strain B6C3F1[b]	
0 (pooled)	17/92 (18%)	3/78 (4%)
0 (matched)	2/18 (11%)	0/19 (0%)
29.9	16/48 (33%)	NA
30.1	NA	3/47 (6%)
56.2	43/49 (88%)	NA
63.8	NA	34/49 (69%)

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[a] IRDC, 1973a; tumor incidence as determined by Reuber. Tumor incidences significantly different from controls for 25 and 50 ppm in the diet ($p<1\times10^{-8}$) (Epstein, 1976)

[b] NCI (1977a), dose-related trend significant at p<0.0001

NA = Not applicable

__Research Institute for Animal Science in Biochemistry and Toxicology, Japan (RIASBT, 1983a)__ -- In this study, conducted for the Velsicol Chemical Corporation, technical grade chlordane (distribution of isomers not specified) was fed to groups of 80 male and 80 female ICR mice at levels of 0, 1, 5 or 12.5 ppm (~0, 0.14, 0.71 or 1.79 mg/kg bw) for a period of 24 months.

Each group (sex and dose level) consisted of 80 mice of which 8 were sacrificed at 52 weeks. There was no apparent effect of dosing on survival or body weight gain. The mean liver weight was significantly increased for the eight males receiving 12.5 ppm sacrificed at 52 weeks, and the liver-to-body weight ratios were significantly increased for all dosed groups of males when compared with controls. At terminal sacrifice (104 weeks), the mean weight and organ-to-body weight ratio of the liver were statistically significantly increased in both males and females receiving 12.5 ppm. In addition, the liver-to-body weight ratios of females receiving 1 and 5 ppm chlordane were statistically significantly greater than in controls.

A significant increase ($p<0.001$) in the incidence of hepatocellular adenoma and hemangioma of the liver was found in the 12.5 ppm male group in animals dying between 19 and 24 months or at terminal sacrifice, as described in Table V-17. There was no increase in hepatic tumors in female mice. Other than for the liver tumors in male mice, there were no significant differences in tumors at other sites related to chlordane exposure.

TABLE V-17

Neoplastic and Toxic Lesions of the Liver in ICR Mice fed Chlordane[a]

	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	12.5	0	1	5	12.5
No. of tissues examined	71	71	72	72	72	72	71	72
Hepatocellular adenoma	13	13	15	28[b]	1	1	3	1
Hepatocellular adenoma - carcinoma	3	3	7	9	0	0	0	1
Hemangioma	4	1	8	14[b]	0	2	1	0
Hepatocellular swelling and degeneration	5	8	58[b]	59[b]	3	2	24[b]	59[b]
Fatty degeneration	2	0	3	9[c]	1	2	9	9
Necrosis	6	7	23[c]	19[b]	14	8	15	20

[a] Source: RIASBT, 1983a

[b] Statistically significantly different from control value ($p<0.001$)

[c] Significantly different from control value ($p<0.01$)

In addition to liver tumors, there were remarkable increases in liver lesions. There were also significant increases ($p<0.01$) in the incidence of hepatocellular swelling, degeneration and necrosis in males at 5 and 12.5 ppm and a significant increase ($p<0.01$) in fatty degeneration at 12.5 ppm. A significant increase ($p<0.001$) in hepatocellular swelling and degeneration was found in females at doses of 5 and 12.5 ppm. In summary, these liver lesions were observed after 12 months; dose-related increased incidences of hepatocellular swelling and degeneration; fatty degeneration, and necrosis in the livers of males, with a less distinct trend in females.

Studies with Rats.

Ambrose et al. (1953a,b) -- In this study, technical grade chlordane was fed to groups of 3-5 male and female albino rats for 400 days at dietary levels of 0, 10, 20, 40, 80, 160, 320, 640 or 1280 ppm (~0, 0.5, 1.0, 2.0, 4.0, 8.0, 32.0, 64.0 or 128.0 mg/kg bw). Increased mortality was observed in the 640 or 1280 ppm groups, and retarded growth was observed in all animals at ≥320 ppm. No growth retardation was observed in female rats fed ≤160 ppm or in male rats fed 10 or 80 ppm. Significantly enlarged livers and other liver changes were found in male and female rats fed chlordane at ≥80 ppm, and pathologic changes in the liver were occasionally found in male rats fed 40 ppm. No treatment-related increase in tumors was found. The study duration (400 days) is considered too short and the number of animals too small for this to be a valid carcinogenicity study.

Ingle (1952) -- Six groups of 20 male and 20 female Osborne-Mendel rats were fed chlordane for up to 2 years at dietary dose levels of 5, 10, 30, 150 or 300 ppm (~0.25, 0.5, 1.5, 7.5 or 15.0 mg/kg bw). Marked toxicity was encountered at 300 ppm in both males and females. This included high mortality, reduced growth rates, eye and nose hemorrhaging, and severe histopathologic damage to the liver, kidneys, heart, adrenals, lungs, myocardium and spleen. At 150 ppm, similar but less severe effects were seen. The effects at 30 ppm included inducible tremors and slight liver damage. At 10 ppm, only minor liver damage such as occasional hepatocytomegaly and mild bile duct hyperplasia was seen. No symptoms of toxicity, gross or histopathologic changes in the liver, kidneys, lungs, pancreas, testes, ovaries, heart or spleen were noted at 5 ppm. No treatment-related tumor incidence was found.

National Cancer Institute (1977a) -- In the NCI carcinogenicity study, groups of 50 male and 50 female Osborne-Mendel rats were fed chlordane (94.8% pure) in the diet for 80 weeks, at two dose levels -- a predicted MTD and 1/2 MTD. This was followed by a 29-week observation period. As upward or downward adjustments were made in dose levels, the doses are expressed as TWA concentrations. The TWA concentrations for male rats at the low and high doses were 203.5 and 407 ppm (~10.2 and 20.4 mg/kg bw), respectively, and for females, 120.8 and 241.5 ppm (~6.04 and 12.08 mg/kg bw), respectively. Ten rats of each sex served as matched controls, and 60 rats of each sex served as pooled controls. Complete necropsies and histologic examinations were performed, except in the cases of a few spontaneous deaths.

The mean body weight of high-dose males and females was consistently lower than controls. All treated groups had symptoms of toxicity, including loss of body weight, rough and discolored hair, palpable masses, and tumors, that became progressively worse as the study continued. Among female rats, there was a highly significant ($p=0.003$) dose-related increase in mortality. Mortality was not significantly increased for male rats. Only two hepatocellular carcinomas were observed, one in a low-dose male and one among the pooled controls. A significant ($p<0.05$) increase in neoplastic nodules of the liver was seen in the low-dose females but not in the high-dose females or in either the high- or low-dose males. A dose-related trend ($p<0.05$) was found for neoplastic lesions (adenomas and carcinomas) of the thyroid glands (follicular-cell and C-cells) for females when compared with the matched controls. However, the results were ambiguous and internally inconsistent. NCI discounted the importance of these findings because the incidences were comparatively low and were known to be variable in populations of control rats.

In a more recent review of tumors in Osborne-Mendel rats in the NCI studies (>900 of each sex), Goodman et al. (1980) presented data indicating 7.1% follicular cell tumors in control males and 3.4% in control females. These data provide additional support for NCI's decision to discount the importance of an apparent increase in thyroid tumors. A highly significant dose-related increase in the incidence of fibrous histiocytoma ($p=0.0007$) was observed for male rats. This was based on an increase only in the high-dose male group (7/44) as compared with 1/44, 0/8 and 2/58 for the low-dose, matched control and pooled control groups, respectively. The investigators discounted this finding because they did not believe these lesions to be treatment-related, as they had occurred spontaneously throughout the bioassay program. All other tumors were common for this strain of rat, and were not treatment-related.

The Research Institute for Animal Science in Biochemistry and Toxicology, Japan (RIASBT, 1983b) -- In this study, conducted for the Velsicol Chemical Corporation, chlordane (distribution of isomers not specified) was fed to groups of 80 male and 80 female Fischer 344 rats at levels of 0, 1, 5 or 25 ppm (~0, 0.5, 0.25 or 1.25 mg/kg bw) for a period of 130 weeks. Each group (sex and dose level) consisted of 80 rats, of which subsets of eight rats were sacrificed and studied at 26 and 52 weeks. The dose levels were set on the basis of a pilot study in which groups of 5 male and 5 female Fischer 344 rats were fed diets containing 0, 50, 100, 200, 400 or 800 ppm technical grade chlordane for 4 weeks. Hepatocellular swelling and fatty degeneration in the liver were found in both male and female rats at 50 ppm, the lowest dose tested. One ppm was set as the no-effect level, based on an 18-month study in mice (provided by Velsicol) in which changes in the liver were evidenced at 5 ppm chlordane.

In the 130-week study, there were no dose-related effects on mortality, food consumption, water consumption, hematology, clinical chemistry, or urinalysis. Virtually all of the toxic effects were restricted to the liver.

The weight of the liver in females receiving 25 ppm was significantly increased at weeks 26 and 52 but not at week 130, whereas in males receiving 5 and 25 ppm, the liver weight was increased at week 130 but not at the 26- or 52-week sacrifice.

At necropsy, enlargement of the liver was noted in 19 control males and 19, 26, and 32 males dosed at 1, 5 and 25 ppm, respectively. Table V-18 presents the tumor and nontumor lesions of the liver. There was a significant increase in adenomas of the liver in males receiving 25 ppm as compared

TABLE V-18

Liver Tumors and Nonneoplastic Lesions in Fischer 344 Rats Fed Chlordane[a,b]

	Males/dose (ppm)				Females/dose (ppm)			
	0	1	5	25	0	1	5	25
No. of tissues examined	64	64	64	64	64	64	64	64
Hepatocellular adenoma	1	1	3	9[c]	0	2	0	0
Mesenchymoma	0	3	1	2	0	0	0	0
Hepatocellular swelling	5	15[d]	14	42[c]	7	2	8	38[c]
Hepatocellular necrosis	3	13[d]	11	27[c]	0	0	1	1
Hepatocellular fatty degeneration	26	15[d]	19	22	20	16	19	2
Focal hepatocellular hyperplasia	5	7	3	11	5	10	1	1

[a] Source: RIASBT, 1983b

[b] The animals sacrificed at weeks 26 and 52 are not included

[c] Significantly different from control value ($p<0.001$)

[d] Significantly different from control value ($p<0.05$)

with controls, but no corresponding effect occurred in females. All of these tumors were found after 104 weeks (mean time to tumor death was 121.8 weeks). There was also a significant increase in fibroadenomas of the mammary gland in females receiving 1 ppm as compared with controls but no significant increase at 5 or 25 ppm.

Nonneoplastic lesions occurred frequently. There was a dose-related increase in the incidence of hepatocellular swelling and necrosis in male rats. When compared with controls, the incidence of hepatocellular swelling was significantly increased in all dosed males, and the incidence of hepatocellular necrosis was significantly increased in males receiving 1 and 25 ppm. The incidence of hepatocellular swelling was significantly higher in females receiving 25 ppm than in controls. There was also an increase in focal hepatocellular hyperplasia in males receiving 25 ppm, but the increase was not significantly different compared with controls. Most of these lesions of the liver occurred after 78 weeks of the study. A slight increase in nonneoplastic liver lesions was seen in the 26- and 52-week sacrifice groups (Table V-19).

Chlordane was considered positive for oncogenicity by the authors, since the incidence of hepatic adenomas was significantly increased ($p<0.001$) in males in the 25 ppm group (9/64 vs. 1/64 in controls). The historical incidence of this tumor in F344/CRJ rats for the testing laboratory was 2.5% in males and 2.3% in females. The control incidence in this study was 1.6%.

TABLE V-19

Liver Lesions in Fischer 344 Rats Fed Chlordane for 26 or 52 Weeks[a,b]

Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
26 weeks								
Hepatocellular fatty degeneration	0	0	0	1	0	0	1	0
Focal necrosis	0	0	0	0	0	1	0	0
Bile duct proliferation	0	0	0	1	1	0	0	1
52 weeks								
Hepatocellular fatty degeneration	0	2	0	1	0	0	0	0
Hepatocellular swelling	0	0	0	2	0	1	0	3
Focal necrosis	0	0	1	1	0	1	0	1
Bile duct proliferation	0	0	0	0	0	0	1	0
Small granuloma	0	0	0	0	0	0	1	2

[a] Source: RIASBT, 1983b

[b] Livers from eight rats/group were examined.

An independent review of the liver histopathology, conducted by Dr. Gary M. Williams, differed somewhat in that three of the neoplasms of the liver, identified as adenomas by the report authors, were diagnosed as carcinomas by Dr. Williams. In addition, three neoplasms (adenomas) were found that were not diagnosed by the testing laboratory (Table V-20). It was noted that only two slices of liver, one from the median lobe and one from the left lobe, were taken from rats without grossly observed tumors, and Dr. Williams felt that the number of neoplasms might have increased had more sections been evaluated. In his opinion, the small increase in benign liver neoplasms was considered as weak evidence for the oncogenicity of chlordane in rats.

The increased incidence of mammary fibroadenomas in females receiving 1 ppm chlordane was not considered to be compound/dose related because mammary fibroadenomas were absent in females dosed at higher levels.

Nonneoplastic changes in the liver of both males and females were increased in dosed animals compared with controls. The principal changes consisted of hepatocellular swelling and necrosis. The liver changes were accompanied by an increase in liver weights in males receiving 5 and 25 ppm at 130 weeks and in females receiving 25 ppm at weeks 26 and 52.

It was concluded that, under the conditions of the study, technical grade chlordane caused a significant increase in the incidence of benign hepatocellular tumors when fed at a level of 25 ppm in the diet to male F344 rats for 130 weeks. There was also a significant increase in nonneoplastic lesions of the liver in both male and female rats, namely, an increased

TABLE V-20

Liver Neoplasms in Male Fischer 344 Rats Fed Chlordane[a,b]

Neoplasm	Dose (ppm)			
	0	1	5	25
Adenoma	1[c]	1	4	8[c]
Carcinoma	1[c]	0	0	2[c]
Total neoplasms	2	1	4	10[d]

[a] Source: RIASBT, 1983b

[b] Pathology by Gary M. Williams, M.D., dated March 9, 1984

[c] One animal had both an adenoma and carcinoma.

[d] Significantly different from control value ($p \leq 0.05$)

incidence of hepatocellular swelling in males receiving 1, 5 or 25 ppm and in females receiving 25 ppm. In addition, there was a significant increase in hepatocellular necrosis in males receiving 1 or 25 ppm, but no corresponding effect occurred in females. The histologic changes in the liver were accompanied by increased liver weights in males at 130 weeks (5 and 25 ppm groups) and females receiving 25 ppm at weeks 26 and 52. A NOEL and LOEL for chronic toxicity in females based on nonneoplastic changes in the liver are 5 and 25 ppm, respectively; the LOEL in males is 1 ppm.

Other Species.

Wazeter (1967) -- In this unpublished study, Wazeter, as cited in Vettorazzi (1975), performed a chronic study with dogs that were fed a diet containing chlordane at levels of 0, 0.3, 3, 15 or 30 ppm (~0, 0.008, 0.075, 0.375 or 0.75 mg/kg bw) for 2 years. Increased liver weight and histologic changes in the liver were reported along with a NOEL of 3 ppm. No tumors were reported. The study duration (2 years) is considered inadequate for a carcinogenicity assay in dogs.

Animal Studies-Heptachlor -- Heptachlor has been studied in three mouse and five rat long-term carcinogenesis bioassays; Tables V-21 and V-22 present a summary of the experimental design and tumor results for these studies. One long-term chronic study using dogs has also been conducted. These studies are described in more detail in the following sections.

TABLE V-21

Summary of Mouse Dietary Carcinogenicity Tests for Heptachlor and Heptachlor Epoxide

Strain	No./Sex	Dose (ppm)	Duration of Exposure/ Observation	Tumor Results	Reference
C3H	100/M&F	10	2 years	Benign liver tumors (FDA); hepatocellular carcinomas in both males and females (Reuber)	FDA (Davis, 1965); Reuber, 1977b
B6C3F1	50/M&F	6.1 and 13.8, M; 9 and 18, F	80 weeks plus 10 weeks observation	Hepatocellular carcinomas in both males and females	NCI, 1977b
CD-1	100/M&F	1, 5 and 10	18 months observation	Nodular hyperplasia at 5 and 10 ppm in both males and females (IRDC)	IRDC, 1973b
				Hepatocellular carcinomas at 5 and 10 ppm in both males and females (Reuber)	Reuber, as cited in Epstein, 1976

TABLE V-22

Summary of Rat Dietary Carcinogenicity Tests for Heptachlor and Heptachlor Epoxide

Strain	No./Sex	Dose	Duration of Exposure/ Observation	Tumor Results	Reference
Wistar	95/M&F no. of each sex in dose group not specified	10 mg/kg (5 doses)	5 daily doses then observation for 106-110 weeks	No treatment-related increase in tumors	Cabral et al., 1972
Osborne-Mendel	50/M&F	38.9 and 77.9 ppm, M; 25.7 and 51.3 ppm, F	80 weeks plus 30 weeks observation	No liver tumors; neoplastic nodules in treated and control groups; thyroid tumors in females	NCI, 1977b
CD	25/M&F	5, 7.5, 10 and 12.5 ppm	2 years	No increase in tumors; some liver lesions at 7.5 ppm and above	Kettering (Jolley et al., 1966)
CF	20/M&F	1.5, 3, 5, 7 and 10 ppm	110 weeks	No dose-related increase liver lesions at 7 and 10 ppm	Kettering (Witherup et al., 1955)
CFN	25/M&F	0.5, 2.5, 5.0, 7.5 and 10 ppm	108 weeks	No dose-related increase in tumors (Kettering); slight increase in liver nodules and carcinomas (Reuber)	Kettering (Witherup et al., 1959); Epstein, 1976

Studies with Mice.

The Food and Drug Administration (Davis, 1965) -- The carcinogenicity of heptachlor and heptachlor epoxide (purity not specified) was studied using groups of 100 male and 100 female C3H mice fed a diet mixture containing 0 or 10 ppm (~1.43 mg/kg bw) for 2 years. Survival was generally low: 50% of the controls, 30% of the heptachlor-treated, and 9.5% of the heptachlor epoxide-treated mice survived the 2-year period; early deaths were due to tumors. FDA pathologists found a 2-fold increase in benign liver lesions (hepatic hyperplasia and benign tumors) in the treated animals over the controls, although the incidence of malignant liver tumors was less (Table V-23).

A reevaluation was performed by Reuber (1977b), resulting in a change in diagnosis for many benign tumors to liver carcinomas. Hepatic carcinomas in the treated groups were generally large, especially in the epoxide groups, and were frequently multiple, in contrast to carcinomas in control groups, which were smaller and solitary. The incidences of liver carcinoma are presented in Table V-24. Reuber's findings yielded a highly significant (p=5x10^{-}[illegible] to <1x10^{-}[illegible]) increase in incidences of liver carcinoma in both sexes for heptachlor and heptachlor epoxide. Four other independent pathologists (Drs. R. Squire, R. Sternberg, H. Stewart and G. Williams) reviewed a sample of 19 slides and generally concurred in the Reuber diagnoses (Epstein, 1976).

In addition to liver tumors, Reuber also diagnosed nontumor liver lesions, primarily hyperplasia, nodules, hepatic vein thrombosis, and cirrhosis, in the heptachlor and heptachlor epoxide-treated mice.

TABLE V-23

Incidences of Liver Lesions in C3H Mice Treated with Heptachlor or Heptachlor Epoxide[a]

Dose Group	Hepatic Hyperplasia	Benign Liver Tumors Only	Malignant Liver Tumors Only
Controls	38/200	30/200	21/200
Heptachlor (10 ppm)	108/200	51/200[b]	10/200
Heptachlor epoxide (10 ppm)	65/200	85/200[c]	13/200

[a] Source: Davis, 1965

[b] Statistically significant ($p<10^{-}$[illegible])

[c] Statistically significant ($p<0.0064$)

TABLE V-24

Incidences of Hepatocellular Carcinoma in C3H Mice Treated with Heptachlor or Heptachlor Epoxide*

Dose group	Males	Females
Controls	22/78 (28%)	2/54 (4%)
10 ppm heptachlor	64/87 (73%)	57/78 (73%)
10 ppm heptachlor epoxide	73/79 (92%)	77/81 (95%)

*Source: Davis, 1965 (as diagnosed by Reuber, 1977b)

National Cancer Institute (1977b) -- In the NCI carcinogenicity bioassay conducted at Gulf South Research Institute, technical grade heptachlor (~73% heptachlor, 22% trans-chlordane and 5% nonochlor) was fed to groups of 50 B6C3F1 mice of each sex for 80 weeks, followed by an observation period of 10 weeks. The TWA high-dose and low-dose concentrations were 6.1 and 13.8 ppm (~0.87 and 1.97 mg/kg bw) for males and 9.0 and 18.0 ppm (~1.29 and 2.57 mg/kg bw) for females, respectively. Controls consisted of 20 male and 10 female matched control mice, and 100 male and 80 female pooled control mice.

Hepatocellular carcinoma was the most frequently observed neoplasm. The incidence in high-dose males was significantly higher ($p=0.001$) when compared with matched controls, while the incidence in low-dose males was comparable to that of the control groups. The difference in hepatocellular carcinoma incidence between high-dose females and matched controls was also significant ($p<0.005$), and there was a highly significant ($p<0.0001$) dose-related increase in the incidence of hepatocellular carcinomas for female mice due to the difference between the high- and low-dose groups. Other tumor types were observed with low frequency among all groups. The incidence of hepatocellular carcinoma in mice is presented in Table V-25. There were also many nodules in the mice that did not have liver carcinomas.

International Research and Development Corporation (1973b) -- IRDC, under contract with Velsicol Chemical Corporation, fed a 25:75 mixture of heptachlor:heptachlor epoxide in the diet, at concentrations of 1, 5 or 10 ppm (~0.14, 0.71 or 1.43 mg/kg bw) for 18 months to groups of 100 male and 100 female CD-1 mice, starting at 7 weeks of age. Similar control groups

TABLE V-25

Incidence of Hepatocellular Carcinoma in B6C3F1 Mice Following Chronic Dietary Exposure to Heptachlor/Chlordane Mixture[a,b]

Groups (ppm diet)	Incidence[b]
MALES	
0 (pooled)	17/92 (18%)
0 (matched)	5/19 (26%)
6.1	11/46 (24%)
13.8	34/47 (72%)[c]
FEMALES	
0 (pooled	3/78 (4%)
0 (matched)	2/10 (20%)
9.0	3/47 (6%)
18.0	30/42 (71%)[d]

[a] Source: NCI, 1977b

[b] Incidence expressed as $\frac{\text{No. of tumor-bearing mice}}{\text{No. of tissues examined}}$

[c] Statistically different from matched controls (p=0.0001); also dose-related increase in male mice (p<0.0001).

[d] Statistically different from matched controls (p<0.005), also dose-related increase in female mice (p<0.0001).

were fed an insecticide-free diet. The incidences of hepatomas were lower among the higher dose groups than in the 1 mg/kg diet group and controls. However, the incidences of nodular hyperplasia (Table V-26) were highly significant at 5 and 10 ppm in both males and females when compared with controls. Upon reexamination of the slides, Reuber diagnosed more hepatic carcinomas and less hyperplasia and hyperplastic nodules (Epstein, 1976). Five other pathologists (Drs. J. Rust, P. Newberne, R. Squire, H. Stewart and G. Williams) examined a portion of the slides and agreed with Reuber that the incidence of hepatic carcinoma was considerably underdiagnosed in the original analysis (Epstein, 1976). The incidences of liver carcinoma as diagnosed by Reuber are presented in Table V-27.

Studies with Rats.

Cabral et al. (1972) -- Heptachlor (96.8% pure) in corn oil was administered by gavage to Wistar rats in five doses of 10 mg/kg bw each on alternate days starting at 10 days of age. The heptachlor-treated group contained 95 Wistar rats, 7 of which died before weaning. The controls consisted of 19 males and 27 females treated with corn oil alone. Many rats were lost because of high mortality of both treated and control rats and to an interim sacrifice at 60 weeks. Twenty-nine females and 30 males remained after 60 weeks and comprised the carcinogenicity test groups. All surviving rats were sacrificed at 106-110 weeks. There was no indication of a treatment-related increase in tumors.

Kettering Laboratory (Jolley et al., 1966) -- In this study, a 75:25 mixture of heptachlor:heptachlor epoxide was administered to groups of 25 female CD rats in the diet at concentrations of 5, 7.5, 10 and 12.5 ppm

TABLE V-26

Incidence of Nodular Hyperplasia in CD-1 Mice Exposed to Heptachlor/Heptachlor Epoxide (25:75) Mixture*

Dose (ppm)	Males	Females
0	0/50 (0%)	1/67 (1%)
1	2/53 (4%)	0/63 (0%)
5	24/57 (42%)	9/56 (16%)
10	53/69 (77%)	28/46 (61%)

*Source: IRDC, 1973b

TABLE V-27

Incidence of Hepatic Carcinoma in CD-1 Mice Following Chronic Dietary Exposure to Heptachlor:Heptachlor Epoxide (25:75)[a]

Groups (ppm diet)	Incidence[b]
MALES	
0	0/62 (0%)
1.0	2/68 (3%)
5.0	18/61 (26%)
10.0	52/80 (65%)[c]
FEMALES	
0	6/76 (8%)
1.0	1/70 (10%)
5.0	6/65 (9%)
10.0	30/57 (53%)[c]

[a] Based on Reuber's reevaluation of IRDC (1973b) slides (Epstein, 1976)

[b] Incidence expressed as $\frac{\text{No. of tumor-bearing mice}}{\text{No. of tissues examined}}$

[c] Significantly different from control value ($p \leq 0.001$).

(~0.25, 0.375, 0.5 and 0.625 mg/kg bw) for 2 years. A dose-related increase in mortality was observed. A comprehensive histological evaluation revealed spontaneous tumors, such as mammary adenomas or fibroadenomas, with random frequency among treatment and control groups. No malignant lesions of the liver were observed, although hepatocytomegaly was increased at 7.5, 10 and 12.5 ppm.

National Cancer Institute (1977b) -- In the NCI bioassay conducted at Gulf South Research Institute, technical grade heptachlor was fed for 80 weeks to groups of 50 male and 50 female Osborne-Mendel rats at a TWA dietary level of 38.9 or 77.9 ppm (~1.95 or 3.90 mg/kg bw) for males and 25.7 or 51.3 ppm (~1.29 or 2.57 mg/kg bw) for females. Animals were maintained for an additional 30 weeks on a heptachlor-free diet. Ten rats of each sex served as the matched controls, and 60 rats of each sex served as pooled controls.

Overall, the incidence of neoplastic liver lesions was somewhat greater in the heptachlor-treated groups than that observed after chlordane treatment. However, no hepatocellular carcinomas were observed in any of the rats, with one cholangiocarcinoma diagnosed in one low-dose male. Neoplastic nodules were observed in all treated and control groups, with no statistically significant ($p \leq 0.05$) dose-related trend. The incidences are presented in Table V-28. A statistically significant exact test for a dose-related trend ($p<0.002$) was found for follicular-cell carcinomas of the thyroid of females, but not in males, when they were combined with adenomas.

TABLE V-28

Incidences of Neoplastic Nodules in Osborne-Mendel Rats Following Chronic Dietary Exposure to Heptachlor[a,b]

Groups (ppm diet)	Incidence[c]
MALES	
0 (pooled)	2/58 (3%)
0 (matched)	1/10 (10%)
38.9	3/44 (7%)
77.9	6/49 (12%)
FEMALES	
0 (pooled)	5/59 (8%)
0 (matched)	1/10 (10%)
25.7	9/48 (19%)
51.3	5/46 (11%)

---

[a] Source: NCI, 1977a

[b] Not significant ($p \leq 0.05$) by either exact test or life-table adjustment

[c] Incidence expressed as $\frac{\text{No. of nodule-bearing rats}}{\text{No. of tissues examined}}$

However, this finding was discounted by NCI because the incidences of carcinomas were low and because of the variability of thyroid tumors in control rat populations. The more recent review by Goodman et al. (1980) of historical control tumor incidences in the Osborne-Mendel rat used in the NCI studies lends further support to the NCI decision.

Kettering Laboratory (Witherup et al., 1955) -- In this first Kettering study, heptachlor (purity not specified) was administered to groups of 20 male and 20 female CF rats at dietary levels of 1.5, 3, 5, 7 and 10 ppm (~0.075, 0.15, 0.25, 0.35 and 0.5 mg/kg bw) for 110 weeks. Similar groups of 20 rats served as controls. Benign and malignant tumors were randomly distributed among test and control groups, with greater incidences observed for females, especially at 5 and 7 ppm. Liver lesions, described as the "chlorinated hydrocarbon" type, were observed with high incidence at 7 and 10 ppm in the diet, but no liver lesions were found at lower dose levels. The authors did not believe that the liver lesions were neoplastic. The analysis indicated that the incidence of tumors in treated rats was not significantly different from control incidence.

Kettering Laboratory (Witherup et al., 1959) -- In the second Kettering study, heptachlor epoxide (purity not specified) was administered in the diet to groups of 25 male and 25 female CFN rats at concentrations of 0.5, 2.5, 5.0, 7.5 and 10 ppm (~0.025, 0.125, 0.25, 0.375 and 0.5 mg/kg bw) for 108 weeks. Similar groups of controls were maintained on heptachlor epoxide-free diets. In the Kettering analysis, malignant and benign tumors occurred randomly among the test groups and were not related to heptachlor epoxide treatment. A reexamination of the histologic slides was conducted by two pathologists, Drs. M. Reuber and G. Williams. Dr. Reuber concluded that the incidence of hepatic carcinomas was significantly increased above control incidence at 5 and 10 ppm in female rats. Dr. Williams found more hepatic nodules at the 10 ppm level in males. Table V-29 presents a summary of Reuber's and Williams' diagnoses of liver carcinomas and nodules. Three other pathologists reviewed the Kettering studies and also diagnosed more carcinomas than reported by Witherup (Epstein, 1976).

Studies with Dogs.

Kettering Laboratory (U.S. EPA, 1977) -- In this study, groups of two males and three females were exposed to dietary doses of 0, 0.5, 2.5, 5 and 7.5 ppm heptachlor epoxide (purity not indicated) for 60 weeks, at which time they were sacrificed and autopsied. No tumors were reported. While the liver weights of both males and females tended to increase logarithmically in proportion to the amounts of heptachlor epoxide in the diet, only one male at the highest dose had observable hepatic damage. The damage was characterized by cloudy swelling of the cells with slight clumping of the cytoplasm. The study duration (60 weeks) is considered too short and the number of animals too small for this to be a valid carcinogenicity study.

Mutagenicity.

Chlordane -- Chlordane (unspecified purity, technical grade and commercial formulations) has been tested for mutagenicity and related effects in a number of systems (Table V-30). Negative results have been obtained in reverse mutation assays using nine strains of Salmonella typhimurium and two strains of Escherichia coli with and without metabolic activation systems (Probst et al., 1981; Gentile et al., 1982). Chlordane was negative when

TABLE V-29

Incidence of Hepatic Carcinoma and Neoplastic Nodules in CFN Rats Following Chronic Dietary Exposure to Heptachlor Epoxide[a,b]

Dose (ppm)	Reuber		Williams	
	Carcinomas	Nodules	Carcinomas	Nodules
MALES				
0	1/24 (4%)	6/24 (25%)	0/24 (0%)	0/24 (0%)
0.5	1/22 (5%)	6/22 (27%)	1/23 (4%)	0/23 (0%)
2.5	0/19 (0%)	7/19 (37%)	0/21 (0%)	0/21 (0%)
5.0	1/23 (4%)	9/23 (39%)	1/22 (5%)	0/22 (0%)
7.5	1/25 (4%)	2/25 (8%)	0/25 (0%)	1/25 (4%)
10.0	4/22 (18%)	7/22 (32%)	1/22 (5%)[b]	4/22 (18%)[c]
FEMALES				
0	0/17 (0%)	7/17 (41%)	0/17 (0%)	4/17 (24%)
0.5	3/22 (14%)	8/22 (36%)	0/20 (0%)	5/20 (25%)
2.5	3/18 (17%)	4/18 (22%)	0/18 (0%)	2/18 (11%)
5.0	7/22 (32%)[d]	10/22 (45%)[d]	1/22 (5%)	2/22 (9%)
7.5	3/21 (14%)	14/21 (67%)	0/23 (0%)	5/23 (22%)
10.0	5/19 (26%)[d]	8/19 (42%)[d]	1/19 (5%)	1/19 (5%)

[a] Source: Witherup et al., 1959. Data from the Reuber/Williams diagnoses presented in Epstein, 1976.

[b] Incidence is expressed as $\frac{\text{No. of tumor-bearing rats}}{\text{No. of rats examined}}$

[c] Statistically significant (p=0.019) for combined incidence of carcinomas and nodules (Epstein, 1976).

[d] Statistically significant (p=0.05) for combined incidence of carcinomas and nodules (Epstein, 1976).

TABLE V-30

Mutagenicity Testing of Chlordane

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	*Salmonella typhimurium* G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, TA98	unspecified purity	plate incorporation	four gradient plates to give a 10-fold concentration range/ plate up to 10,000-fold range	±S9	-	- in all tester strains at all concentrations	Probst et al., 1981
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	commercial formulation	plate incorporation	NR	±S9	-	- in all strains	Gentile et al., 1982
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	technical grade	plate incorporation	NR	±S9	-	- in all strains	Gentile et al., 1982
Reverse mutation	*Escherichia coli* WP2, WP2 urvr	unspecified purity	plate incorporation	four gradient plates to give 10-fold concentration range/plate up to 10,000-fold range	±S9	-	- in both strains at all concentrations	Probst et al., 1981
Mitotic gene conversion	*Saccharomyces cerevisiae* D4	commercial formulation	incorporated into log-phase cell suspension	NR	±S9	±	+ with metabolic activation in a dose-related manner	Gentile et al., 1982
Mitotic gene conversion	*S. cerevisiae*	technical grade	incorporated into log-phase cell cell suspension	NR	±S9	±	+ with S9 activation in a dose-related manner	Gentile et al., 1982
Reverse mutation	*Zea mays* W22 homozygous for wx-C allele	commercial formulation	applied prior to seedling emergence	2.24 kg/ha	NA	+	none	Gentile et al., 1982

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TABLE V 30 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
UDS	adult rat primary hepatocytes	purity unspecified	cell culture incorporation	8 doses 0.5-1000.0 nmoles/mℓ	NA	-	chlordane was toxic to cells at >100 nmoles/mℓ	Probst et al., 1981
UDS	adult male rat primary hepatocytes	purity unspecified	applied to cover-slip cell cultures	10^{-8}-10^{-3} M	NA	-	chlordane was toxic to cells at >10^{-4} M	Maslansky and Williams, 1981
UDS	adult male mouse primary hepatocytes	purity unspecified	applied to cover-slip cell cultures	10^{-8}-10^{-3} M	NA	-	chlordane was toxic to cells at >10^{-4} M	Maslansky and Williams, 1981
UDS	adult male hamster primary hepatocytes	purity unspecified	applied to cover-slip cell cultures	10^{-8}-10^{-3} M	NA	-	chlordane was toxic to cells at >10^{-4} M	Maslansky and Williams, 1981
Dominant lethal	mouse CD-1	technical grade	gavage or i.p. injection	0, 50, 100 mg/kg bw	NA	-	- at all concentrations	Arnold et al., 1977
Dominant lethal	mouse CD-1	commercial formulation	gavage or i.p. injection	0, 50, 100 mg/kg bw	NA	-	- at all concentration	Arnold et al., 1977
UDS	SV-40 transformed human fibroblasts (VA-4)	unspecified purity	cell culture incorporation	1, 10, 100 and 1000 µM	±S9	- +	+ at each dose (P<0.05) only without metabolic activation; >3-fold over control at 1 µM	Ahmed et al., 1977
Inhibition of cell growth	Hela cells	Ortho Klor 74 (44.4% chlordane)	cell culture incorporation	0.0075-0.200 µg/mℓ	NA	+	99-100% inhibition with all concentrations tested	Blevins and Sholes, 1978

NR - Not reported; NA - Not applicable; UDS - Unscheduled DNA synthesis; i.p. - intraperitoneal assayed for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures. Concentrations of $>10^{-4}$ M chlordane were toxic to the cells (Probst et al., 1981; Maslansky and Williams, 1981). Commercial and technical grades of chlordane were negative in the dominant lethal assay in CD-1 mice following either intragastric or intraperitoneal administration (Arnold et al., 1977).

Positive results were obtained for mitotic gene conversion assays in *Saccharomyces cerevisiae* with but not without metabolic activation (Gentile et al., 1982). Ahmed et al. (1977) reported a >3-fold increase ($p<0.05$) over controls in unscheduled DNA synthesis by SV-40 transformed human fibroblast cultures (VA-4) treated with 1 μM chlordane, but only in the absence of the S-9 metabolic activating fraction. Ortho-Klor 74 (44.4% chlordane commercial formulation) inhibited by 99-100% the growth of HeLa cells at concentrations of 0.0075-0.200 μℓ/ℓ (Blevins and Sholes, 1978). Finally, chlordane was positive in a reverse mutation assay using a strain of *Zea mays* homozygous for wx-allele (Gentile et al., 1982). This assay in a plant was included for the sake of completeness since it was positive. Further details of these assays are given in Table V-30.

Heptachlor and Heptachlor Epoxide -- Heptachlor has been tested for mutagenicity in bacteria, yeast, maize, fruit flies, mice and rats, and for unscheduled DNA synthesis in mammalian cell cultures (Table V-31). Negative results have been obtained in the reverse mutation assay in 10 strains of *Salmonella typhimurium* and three strains of *Escherichia coli* for heptachlor tested with and without metabolic acitvation (Moriya et al., 1983; Probst et al., 1981; Marshall et al., 1976) and in the rec assay in two strains of *Bacillus subtilis* in which no activating system was used (Shirasu et al.,

TABLE V-31

Mutagenicity Testing of Heptachlor and Heptachlor Epoxide

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	_Salmonella typhimurium_ TA100, TA98, TA1535, TA1537, TA1538	heptachlor/purity NR	plate incorporation	0-5000 μg/plate	±S9	-	- in all strains at all concentrations	Moriya et al., 1983
Reverse mutation	_S. typhimurium_ G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, TA98	heptachlor/NR	plate incorporation	four gradient plates to give 10-fold concentration range/plate up to 10,000-fold range	±S9	-	- in all strains at all concentrations	Probst et al., 1981
Reverse mutation	_S. typhimurium_ TA1535, TA1536, TA1537, TA1538	heptachlor/reference standard pure	plate incorporation	50-1000 μg/plate	±S9	-	- in all strains at all concentrations; toxic level >2500 μg/plate (-S9); >1000 μg/plate (+S9)	Marshall et al., 1976
Reverse mutation	_S. typhimurium_ TA1535, TA1537, TA1538, TA98, TA100	heptachlor/technical grade	plate incorporation	range NR	±S9	±	maximum + in strains TA98 and TA100 with S9 at 10 μg/plate	Gentile et al., 1982
Reverse mutation	_S. typhimurium_ TA1535, TA1537, TA1538, TA98, TA100	heptachlor/commercial formulation	plate incorporation	NR	±S9	-	- in all strains	Gentile et al., 1982
Reverse mutation	_Escherichia coli_ WP2 hcr	heptachlor/NR	plate incorporation	0-5000 μg/plate	±S9	-	None	Moriya et al., 1983
Reverse mutation	_E. coli_ WP2, WP2 uvrA	heptachlor/NR	plate incorporation	four gradient plates to give 10-fold concentration range/plate up to 10,000-fold range	±S9	-	- in both strains at concentrations	Probst et al., 1981

TABLE V-31 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Rec assay	*Bacillus subtilis* H17 Rec⁺, M45 Rec⁻	heptachlor/NR	spot test	NR	none	-	none	Shirasu et al., 1976
Mitotic gene reversion	*Saccharomyces cerevisiae* D4	heptachlor/technical grade	incorporation into log-phase cell suspension	NR	±S9	-	none	Gentile et al., 1982
Mitotic gene reversion	*S. cerevisiae* D4	heptachlor/commercial formulation	incorporation into log-phase cell suspension	NR	±S9	-	none	Gentile et al., 1982
Reverse mutation	*Zea mays* W22 homozygous for wx-c allele	heptachlor/commercial formulation	applied prior to seedling emergence	1.12 kg/ha	NA	+	none	Gentile et al., 1982
Recessive lethal	*Drosophila melanogaster*	heptachlor/NR	injection into abdomen	5×10^{-4}% in water	NA	-	concentration was the highest sub-lethal concentration	Benes and Sram, 1969
UDS	adult rat primary hepatocytes	heptachlor/NR	cell culture incorporation	0.5-1000 nmole/mℓ	NA	-	heptachlor was toxic to cells at >10 nmoles/mℓ	Probst et al., 1981
UDS	adult male rat primary hepatocytes	heptachlor/NR	applied to coverslip cell cultures	10^{-8} to 10^{-3} M	NA	-	heptachlor, was toxic to cells at $>10^{-4}$ M	Maslansky and Williams, 1981
UDS	adult male mouse primary hepatocytes	heptachlor/NR	applied to coverslip cell cultures	10^{-8} to 10^{-3} M	NA	-	heptachlor was toxic to cells at $>10^{-4}$ M	Maslansky and Williams, 1981
UDS	adult male hamster primary hepatocytes	heptachlor/NR	applied to coverslip cell cultures	10^{-8} to 10^{-3} M	NA	-	heptachlor was toxic to cells at $>10^{-4}$ M	Maslansky and Williams, 1981

TABLE V-31 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Dominant lethal	rat/3 generations	heptachlor/NR	diet for 3 generations	1 and 5 ppm (mg/kg diet)	NA	+	increased number of resorbed fetuses	Cerey et al., 1973
Chromosome aberration	rat bone marrow	diet for 3 generations	1 and 5 ppm (mg/kg diet)	NA	NA	+	increased numbers of abnormal mitoses, chromatids, pulverizations and translocations	Cerey et al., 1973
Dominant lethal	mouse CD-1	heptachlor/technical grade	gavage or i.p. injection	3, 7.5 and 15 mg/kg bw	NA	-	- at either dose by either route	Arnold et al., 1977
UDS	SV-40 transformed human fibroblasts	heptachlor/NR	cell cultures incorporation	100, 1000 μM	±S9	±	+ with S9 at both concentrations ($p<0.05$)	Ahmed et al., 1977
Reverse mutation	*S. typhimurium* TA100, TA98, TA1535, TA1537, TA1538	heptachlor epoxide/NR	plate incorporation	0-5000 μg/plate	±S9	-	- for all strains at all concentrations	Moriya et al., 1983
Reverse mutation	*S. typhimurium* TA1535, TA1536, TA1537, TA1538	heptachlor epoxide/ Reference Standard pure	plate incorporation	50-1000 μg/plate	±S9	-	toxic level >2500 μg/plate (-S9); >1000 μg/plate (+S9)	Marshall et al., 1976
Reverse mutation	*E. coli* WP2 hcr	heptachlor epoxide/NR	plate incorporation	0-5000 μg/plate	±S9	-	none	Moriya et al., 1983
Recessive lethal	*D. melanogaster*	heptachlor epoxide	injection into abdomen	2.5×10^{-4}% in water	NA	-	concentration was the highest sublethal concentration	Benes and Sram, 1969

TABLE V-31 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Dominant lethal	mouse CD-1	heptachlor epoxide: heptachlor (75:25)	gavage or i.p. injection	0, 7.5 and 15 mg/kg bw	NA	-	- at either dose by either route	Arnold et al., 1977
UDS	SV-40 transformed fibroblasts	heptachlor epoxide/NR	cell culture incorporation	10, 100, 1000 μM	±S9	±	+ with S9 at each concentration ($p<0.05$)	Ahmed et al., 1977

NR - Not reported

NA - Not applicable

UDS - Unscheduled DNA synthesis

i.p. - intraperitoneal

1976). Gentile et al. (1982), however, reported a maximum positive response for reverse mutation in strains TA98 and TA100 of _S_. _typhimurium_ at 10 μg technical grade heptachlor/plate with S-9 activation. Negative results were obtained in these strains when a commercial formulation of heptachlor was tested. Heptachlor, whether technical or commercial grade, was also negative for mitotic gene reversion in _Saccharomyces_ _cerevisiae_ in the presence and in the absence of S-9 (Gentile et al., 1982). Negative results were obtained in the recessive lethal assay in _Drosophila_ _melanogaster_ (Benes and Sram, 1969) and for unscheduled DNA synthesis by rat, mouse and hamster primary hepatocyte cultures (Probst et al., 1981; Maslansky and Williams, 1981). The dominant lethal assay in CD-1 mice receiving heptachlor either by gavage or intraperitoneal injection also gave negative results (Arnold et al., 1977); however, the dominant lethal assay in rats fed heptachlor at 1 and 5 mg/kg diet for three generations resulted in increased numbers of resorbed fetuses (Cerey et al., 1973). Increased incidences of chromosome aberrations were also noted in the bone marrow of these rats. Ahmed et al. (1977) reported positive results ($p<0.05$) for unscheduled DNA synthesis in SV-40 transformed human fibroblasts (VA-4) with, but not without, the S-9 fraction at 100 and 1000 μM heptachlor.

Heptachlor epoxide was negative in reverse mutation assays in several strains of _S_. _typhimurium_ and _E_. _coli_ with and without metabolic activation (Moriya et al., 1983; Marshall et al., 1976) in the recessive lethal assay in _D_. _melanogaster_ (Benes and Sram, 1969) and in the dominant lethal assay in CD-1 mice (Arnold et al., 1977). Ahmed et al. (1977) reported that heptachlor epoxide was positive in inducing unscheduled DNA synthesis in the SV-40 transformed human fibroblasts at concentrations of 10, 100 or 1000 μM in the presence, but not the absence, of S-9.

Teratogenicity and Other Reproductive Effects.

Chlordane -- Ingle (1952) found no fetotoxic or teratogenic effects in neonatal Osborne-Mendel rats following *in utero* exposure to technical chlordane. Female rats, two each from groups receiving chlordane at continuous dietary levels of 5, 10, 30, 150 or 300 ppm (mg/kg diet), were mated, one during the 24th week and the other during the 48th week of treatment. No effects were noted on fetal mortality, litter size or health of the pups. Three pups from each litter were allowed to suckle from foster dams that had not been exposed to chlordane; another three from each litter remained with their treated mothers. No differences in growth rates of pups resulted from suckling with exposed or non-exposed dams at 5, 10 or 30 mg/kg diet levels. No differences in growth rates were noted when these pups were compared with control pups that had not been exposed either *in utero* or by suckling. Hyperexcitability and mild tremors were present in pups born to and nursed by dams at the 150 mg/kg diet level that were mated at 48 weeks. One male pup died. No symptoms of toxicity developed in equivalent pups nursed by nontreated foster mothers. Of pups born to 300 mg/kg diet level dams, those transferred to non-treated foster dams did not develop toxic symptoms; those remaining with their treated natural mothers (24-week mating) developed tremors after nursing for 4 days. One male and two female sucklings died. One weanling male died and was underweight. Mortality was 100% within 5 days of nursing by dams treated at the 300 mg/kg diet level (48-week mating). Pups born to untreated dams but nursed by treated dams were similar to the pups born to and nursed by dams treated at each respective dietary level.

Welch et al. (1971) studied the effect of chlordane on mouse fertility. Female Swiss-Webster mice were treated intraperitoneally with 25 mg/kg bw technical chlordane in corn oil once a week for 3 weeks. Controls received only corn oil. The mice were mated on the last day of injection and killed before the gestational period was complete. No effect was observed on litter size for pregnancy; however, the number of mice that became pregnant was appreciably reduced. Of 104 control mice, ~40 (38%) became pregnant. Of 105 treated mice, ~17 (16%) became pregnant. Microsomes prepared from ovariectomized mice treated intraperitoneally with 25 mg/kg bw chlordane once a week for 3 weeks increased the metabolism of estradiol by 3-5 times the control level. Chlordane pretreatment also inhibited the in vivo uterotropic action of estrogens 88-98%. Welch et al. (1971) also studied the effects of chlordane pretreatment on estrogenic effects in Sprague-Dawley rats. Female rats (19-20 days of age) were treated intraperitoneally with technical chlordane or transchlordane (>99%) in corn oil daily for 7 days. At 25 mg/kg bw/day of either compound, the in vitro metabolism of estrone-4-^{14}C by hepatic microsomes prepared from the treated rats was significantly increased. Pretreatment with technical grade chlordane at 10 or 50 mg/kg bw/day for 7 days significantly enhanced the in vivo metabolism of intraperitoneally administered estrogens. The uterotropic response of estrone was inhibited 93 and 98% by technical and transchlordane, respectively. The inhibition was significant ($p<0.05$) at ≥ 5 mg/kg bw/day of technical grade chlordane.

Heptachlor and Heptachlor Epoxide -- No data on the teratogenicity of heptachlor or heptachlor epoxide were available. Mestitzova (1967) studied the effect of heptachlor on the fertility of rats. Heptachlor (98.1% pure) was "administered with food" at an "applied dose" of 6 mg/kg bw to an unspecified strain of rats. It is unclear whether this was a daily dose or a total dose administered over the duration of the treatment. There was a marked reduction in litter size in F_1 generations and in one F_2 generation. Suckling pups had a high rate of mortality. During the first week after birth a mean percent mortality of the exposed pups was 46% as compared with 12% in controls. Cataracts developed in treated adults as well as in pups.

Welch et al. (1971) studied the effect of heptachlor on the metabolism of estrogen by Sprague-Dawley rats. Immature female rats (19-20 days of age) were treated intraperitoneally with heptachlor (>99% pure) at 10 mg/kg bw/day for 7 days. The in vitro metabolism of estrone-4-^{14}C by microsomes prepared from the treated rats was significantly enhanced 2.5-fold over control levels. Pretreatment of rats by the same dose schedule inhibited the in vivo uterotropic response of estrone by 67%.

Summary

Chlordane. Values for acute oral LD_{50}s of chlordane range from 83 mg/kg bw for cis-chlordane (Podowski et al., 1979) to 560 mg/kg bw for technical grade chlordane (Ambrose et al., 1953a,b), when administered in lipophilic vehicle. Gaines (1960) determined an oral LD_{50} of 335 mg/kg bw for male and 430 mg/kg bw for female Sherman rats. Mice had about equal sensitivity (LD_{50} = 390 mg/kg bw), while hamsters were much less sensitive (LD_{50} 1720 mg/kg bw) (Gak et al., 1976). Neonatal rats were less sensitive than adults with an intraperitoneal LD_{50} of 1121 mg/kg bw (Harbison, 1975). Cis-chlordane was more toxic than trans-chlordane to mice (Ivie et al., 1972). Symptoms of acute intoxication include CNS stimulation, as evidenced by irritability, tremors and convulsions (Boyd and Taylor, 1969; Stohlman et al., 1950; Hyde and Falkenberg, 1976). At sublethal doses, hepatic microsomal enzyme induction (Den Tonkelaar and Van Esch, 1974) and enhanced activities of hepatic and kidney cortical gluconeogenic enzymes (Kacew and Singhal, 1973) were observed. Delayed onset of eye and vaginal opening occurred in neonatal mice treated subcutaneously with 0.075 mg/mouse (Talamantes and Jang, 1977).

Maximum tolerated subchronic dietary concentrations were 800 and 400 mg/kg diet for male and female Osborne-Mendel rats, respectively, and 40 and 80 mg/kg diet for male and female B6C3F1 mice, respectively (NCI, 1977a). Subchronic dietary exposure to chlordane has resulted in changes in prostate homeostasis in rats (Shain et al., 1977). The no-effect level for microsomal enzyme induction was <5 mg/kg diet (Den Tonkelaar and Van Esch, 1974). Daily oral intake of 1.2 mg/kg bw chlordane by rats resulted in no histopathologic damage to lungs, heart, stomach, liver, kidneys, spleen or testes (DeLong and Ludwig, 1954). Ingle (1952) defined a no-effect level in Osborne-Mendel rats at 5 mg/kg diet chlordane for histopathological changes in liver, kidneys, lungs, pancreas, stomach, adrenals, thyroid, thymus, lymph, testes, ovaries, heart and spleen, as well as for food consumption, growth rate and mortality. A dietary level of 10 mg chlordane/kg diet produced hepatocytomegaly and mild bile duct proliferation. At higher concentrations, progressively more severe histopathological damage to liver and other organs occurred. Increased incidences of hepatic hyperplasia and hepatocytomegaly in mice were reported in a carcinogenicity study (IRDC, 1973a), but histological re-evaluation of slides by independent pathologists indicated that the hyperplastic nodules were carcinomas (Epstein, 1976).

The incidence of liver hyperplasia was not increased in mice or rats in the NCI (1977a) bioassay, but benign proliferative lesions in chlordane-treated mice were found by Becker and Sell (1979).

Four chlordane carcinogenesis bioassays in mice have been reported. The strains tested include C57Bl/6N, CD-1, B6C3F1 and ICR. In C57Bl/6N mice fed 25 or 50 ppm for 18 months, hepatocellular carcinomas were observed in 27% (16) of the survivors. This mouse strain rarely develops spontaneous liver lesions. For CD-1 mice fed 5, 25 or 50 ppm for 18 months, liver nodules/hepatocellular carcinomas were observed in the 25 and 50 ppm groups. In B6C3F1 mice fed ~30 and 60 ppm for 80 weeks and then held for 10 weeks, hepatocellular carcinomas were observed in both males and females. For ICR mice fed 1, 5 or 12.5 ppm for 24 months, hepatocellular adenomas and hemangiomas were significantly increased ($p<0.001$) in males receiving 12.5 ppm and nonneoplastic liver lesions were present in males fed 5 ppm and in females fed 5 or 12.5 ppm.

Four chlordane carcinogenesis bioassays in rats have been reported. The strains tested include albino, Osborne-Mendel and Fischer 344. Three of these studies were considered adequate and one was inadequate. In albino rats fed 10, 20, 40, 80, 160, 320, 640 or 1280 ppm for 400 days, there were no treatment-related tumors. In Osborne-Mendel rats fed 5, 10, 30, 150 or 300 ppm for 2 years, hepatic toxicity was noted at 150 and 300 ppm, but no liver tumors were noted. In Osborne-Mendel rats fed 203.5 or 407 ppm (males) or 120.8 or 241.5 ppm (females), respectively, for 80 weeks and held for an additional 29 weeks, no liver tumors were noted, but thyroid tumors were significantly increased. In light of historical data for Osborne-Mendel rats, the thyroid tumors were not considered to be treatment-related.

In Fischer 344 rats fed 1, 5 or 25 ppm for 130 weeks, there was a statistically significant increase in hepatocellular adenomas, which was considered by the authors as weak evidence for carcinogenicity in males fed 25 ppm. Hepatocellular swelling was significant in females fed 25 ppm. The hepatocellular adenomas occurred only in males surviving longer than 104 weeks.

Negative results for mutagenicity of chlordane were obtained in nine strains of _Salmonella typhimurium_ and two strains of _Bacillus subtilis_ for reverse mutation with or without metabolic activation (Probst et al., 1981; Gentile et al., 1982); in rat, mouse and hamster primary hepatocyte cultures for unscheduled DNA synthesis (Probst et al., 1981; Maslansky and Williams, 1981); and in mice for the dominant lethal assay (Arnold et al., 1977). Positive results were obtained in _Saccharomyces cerevisiae_ for mitotic gene conversion with, but not without, metabolic activation (Blevins and Sholes, 1978) and in maize for reverse mutation (Gentile et al., 1982).

Ingle (1952) found no fetotoxic or teratogenic effects in neonatal Osborne-Mendel rats born to dams that were continuously fed chlordane at 5-300 mg/kg diet. Pups born to and nursed by treated dams developed dose-related symptoms of toxicity. Pups nursed by non-treated dams did not develop toxic signs. Pups born to non-treated dams and nursed by treated dams were comparable with pups born to and nursed by treated dams. Welch et al. (1971) found that approximately half as many mated female mice, which had been treated intraperitoneally with three weekly doses of 25 mg/kg bw/week of chlordane, became pregnant, as did untreated mice. Chlordane pretreatment in mice and rats resulted in enhanced _in vitro_ and _in vivo_ metabolism of estrogen and inhibited the uterotropic response to estrogen.

Heptachlor and Heptachlor Epoxide. Heptachlor is more toxic than chlordane to laboratory animals. Acute oral LD_{50} values in rats for heptachlor range from 40 mg/kg bw for a commercial formulation (Ben Dyke et al., 1970) to 162 mg/kg bw for female Sherman rats for technical grade heptachlor (Gaines, 1960). Oral LD_{50}s were 70 mg/kg bw for mice and 105 mg/kg bw for hamsters (Gak et al., 1976). The intraperitoneal LD_{50} for neonatal rats was 531 mg/kg bw, but was reduced to 133 mg/kg bw by pretreatment with phenobarbital. It was suggested that the enhanced metabolic capacity of the neonate after phenobarbital dosing resulted in the metabolism of heptachlor to the more toxic heptachlor epoxide (Harbison, 1975). The acute oral LD_{50} for heptachlor epoxide in adult rats was ~60 mg/kg bw (NAS, 1977; Sperling and Ewinike, 1969; Podowski et al., 1979). Symptoms of acute intoxication include tremors, convulsions, paralysis and hypothermia (Hrdina et al., 1974; Yamaguchi et al., 1980).

Moderate liver damage, accompanied by increased levels of serum GPT and serum aldolase, was observed in rats following single (60 mg/kg bw) or repeated oral doses (7 or 12 mg/kg bw/day) for 3-14 days (Krampl, 1971); however, tolerance appeared to develop at 28 days. Enhanced activities of gluconeogenic enzymes, such as pyruvate carboxylase and glucose-6-phosphatase, in rats treated with a 200 mg/kg bw dose (above the LD_{50}) was observed by Kacew and Singhal (1973). Dietary levels of 2-50 mg/kg diet for 14 days resulted in induction of hepatic microsomal enzymes (Den Tonkelaar and Van Esch, 1974).

Maximum tolerated subchronic dietary concentrations were 160 and 80 mg/kg diet for male and female Osborne-Mendel rats, respectively, and 40 mg/kg diet for male and female $B6C3F_1$ mice (NCI, 1977b). Subchronic dietary exposure to heptachlor has resulted in changes in prostate homeostasis in rats (Shain et al., 1977) and induction of hepatic microsomal enzymes (Kinoshita and Kempf, 1970).

A dose-related increase in liver weight in male and female mice treated with 1-10 mg/kg diet levels of a heptachlor:heptachlor epoxide (25:75) mixture was reported in an unpublished carcinogenicity study (IRDC, 1973b, reviewed by Epstein, 1976). Increased incidences of hepatocytomegaly and hyperplastic nodules were also reported to be significantly increased. The histologic slides from this study were re-evaluated by six independent pathologists. The overall interpretation was that many of the hyperplastic nodules diagnosed by IRDC (1973b) were actually carcinomas (Epstein, 1976). Epstein (1976) examined other unpublished reports of heptachlor and heptachlor epoxide carcinogenicity (Davis, 1965; Witherup et al., 1959) that reported increased incidences of hepatic hyperplasia or nodules. Reevaluations by independent pathologists again resulted in an interpretation in favor of increased incidences of carcinomas rather than hyperplastic nodules. Other unpublished reports (Witherup et al., 1955; Jolley et al., 1966) were reviewed by Epstein (1976) and describe increased incidences of hepatocytomegaly and hepatic hyperplasia in rats and mice after chronic feeding with heptachlor and heptachlor epoxide. The incidence of liver hyperplasia was not increased in mice or rats in the NCI (1977b) bioassay. Decreased body weight gain was observed in male and female rats treated at 77.9 and 51.3 mg/kg diet levels, respectively. Reuber (1977a), in re-evaluating the slides from the Davis (1965) study, found appreciable incidences of hepatic vein thrombosis and cirrhosis of the liver in male and female mice treated at 10 mg/kg diet levels of heptachlor or heptachlor epoxide for 2 years. These conditions were not observed in any of the 127 controls.

Epstein (1976) reviewed several unpublished reports of chronic dietary carcinogenicity studies of heptachlor and heptachlor epoxide in rats and mice (Davis, 1965; Witherup et al., 1955, 1959; Jolley et al., 1966; IRDC, 1973b) in an article based on a Statement of Suspension Testimony at EPA Hearing on Heptachlor/Chlordane. Epstein (1976) also presented results of independent statistical analyses and re-evaluations of histologic slides. Increased incidences of nodular hyperplasia were reported for mice after heptachlor or heptachlor epoxide exposure (Davis, 1965; IRDC, 1973b). Independent re-evaluation of slides resulted in statistically significant increased incidences of hepatocellular carcinoma in mice and rats (Epstein, 1976). The dietary levels of heptachlor or heptachlor epoxide in these studies were 0.5-12.5 mg/kg diet.

Three heptachlor/heptachlor epoxide carcinogenesis bioassays in mice have been reported. The strains studied include C3H, B6C3F1 and CD-1 mice. In C3H mice fed 10 ppm of both heptachlor and heptachlor epoxide for 2 years, benign liver tumors/hepatocellular carcinomas were reported in both male and female mice. Hepatocellular carcinomas in treated groups were generally large and frequently multiple tumors, especially in the epoxide group in respect to the controls. For B6C3F1 mice fed technical grade (containing 22% chlordane) at concentrations of 6.1 or 13.8 ppm (males) or 9 or 18 ppm (females), respectively, for 80 weeks and held for an additional 10 weeks, hepatocellular carcinomas were significantly ($p<0.001$) increased in both male and female mice. In CD-1 mice fed a mixture of heptachlor epoxide/heptachlor (75:25) at concentrations of 1, 5 or 10 ppm for 18 months, nodular hyperplasia/hepatocellular carcinomas were noted at 5 and 10 ppm in both male and female mice.

Five heptachlor/heptachlor epoxide carcinogenesis bioassays in rats have been conducted. The strains of rats studied include Wistar, Osborne-Mendel, CD and CFN. In Wistar rats given 5 doses of 10 mg/kg bw of heptachlor and held for 106 to 110 weeks, no treatment-related tumors were observed. For Osborne-Mendel rats fed technical grade heptachlor at concentrations of 38.9 or 77.9 (males) or 25.7 or 51.3 (females) ppm, respectively, for 80 weeks and held for 30 weeks, no liver tumors were noted, although neoplastic nodules were found in both treated and control rats. In CD rats fed a mixture of heptachlor/heptachlor epoxide (75:25) at concentrations of 5, 7.5, 10 or 12.5 ppm for 2 years, no liver tumors were noted, although nonneoplastic lesions were noted in the livers of rats fed 7.5, 10 or 12.5 ppm. In one study using CFN rats fed 1.5, 3, 5, 7 or 10 ppm of heptachlor for 110 weeks, the incidence of liver tumors was not statistically different in treated and control animals. In a second study using CFN rats fed 0.5, 2.5, 5, 7.5 or 10 ppm of heptachlor epoxide for 108 weeks, treatment-related liver carcinomas were noted by several pathologists.

Heptachlor was negative for reverse mutation in 10 strains of *Salmonella typhimurium* and 3 strains of *Escherichia coli* with and without metabolic activation (Moriya et al., 1983; Probst et al., 1981); in 2 strains of *Bacillus subtilis* in the rec assay (Shirasu et al., 1976); in *Saccharomyces cerevisiae* for mitotic gene conversion in the presence of S-9 (Gentile et al., 1982); in *Drosophila melanogaster* for recessive lethality (Benes and Sram, 1969); for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures (Maslansky and Williams, 1981); and for the dominant lethal assay in mice (Arnold et al., 1977). Positive results were obtained in the dominant lethal assay in rats (Cerey et al., 1973) and for unscheduled DNA synthesis in SV-40 transformed human fibroblasts with metabolic activation (Ahmed et al., 1977).

Heptachlor epoxide was negative for reverse mutations in S. typhimurium and E. coli with and without metabolic activation (Moriya et al., 1983; Marshall et al., 1976); recessive lethal assay in D. melanogaster (Benes and Sram, 1969); and the dominant lethal assay in mice (Arnold et al., 1977). Heptachlor epoxide was positive for unscheduled DNA synthesis in SV-40 transformed human fibroblast cultures (Ahmed et al., 1977).

Heptachlor was reported to cause a marked reduction in litter size of rats when administered in the diet for several generations (Mestitzova, 1967). The dose in this study was not clearly defined.

VI. HEALTH EFFECTS IN HUMANS

Clinical Case Studies

Many clinical case studies describing the acute and chronic toxic effects of technical grade chlordane, containing heptachlor, are available in the literature. The three effects seen most frequently are CNS effects, blood dyscrasias and neuroblastoma. In an attempt to substantiate the toxicity associated with chlordane exposure in clinical case studies, Dwight DeLong subjected himself twice, during two separate time intervals, to 7% chlordane vapors for 15 minutes every 3 days for 12 weeks (DeLong and Ludwig, 1954). No treatment-related effects were detected during repeated medical examinations, described as thorough but not further specified by the authors.

CNS Effects Associated with Exposure to Chlordane and Heptachlor. Ten clinical case studies describing CNS effects, along with miscellaneous other effects, following oral, dermal and inhalation exposure to technical grade chlordane containing heptachlor are summarized in Table VI-1. These effects frequently included, but were not limited to, irritability, salivation, labored respiration, muscle tremors, convulsions and death with or without an immediately preceding period of deep depression.

Five of these case studies described toxic effects occurring after oral exposure. Several CNS effects were observed in a 32-year-old woman (Case No. 1) who had ingested 104 mg chlordane/kg bw (Derbes et al., 1955), and in an 18-year-old woman (Case No. 2) who had ingested 32 mg chlordane/kg bw (estimated to be 10 mg/kg after vomiting) (Dadey and Kammer, 1953). Similar effects on the CNS were observed following ingestion of estimated chlordane

TABLE VI-1

Summary of Cases with CNS Effects Associated with Exposure to Chlordane or Heptachlor, Either Alone or in Combination with Other Agents

Case No.	Case Description	Route of Exposure	Dose	Formulation	Duration of Exposure	Possible Confounding Factors	Symptoms	Reference
1	32-year-old, chronically depressed, white, widowed woman, weighing ~58 kg	oral (ingestion)	6 g chlordane (104 mg/kg bw)	toxichlor dust containing 5% chlordane and related chemicals and 95% talc	single exposure	none	Clinical symptoms included chemical burns of the mouth, severe hemorrhagic gastritis, shock, anuria, convulsions, death; at autopsy, pathological findings included cerebral congestion and edema, lower nephron nephrosis, confluent bronchopneumonia and uremia.	Derbes et al., 1955
2	18-year-old, white, female student weighing ~50 kg	oral (ingestion)	1.6 or 32 mg/kg (estimated that 500 mg or 10 mg/kg bw was retained after vomiting)	40% oil solution containing 24% chlordane	single exposure	none	Starting at 1.5 hours after ingestion: nausea, vomiting, diplopia, blurred vision, twitching of the extremities, generalized convulsion, no abnormal blood or urinalysis values, costovertebral angle tenderness.	Dadey and Kammer, 1953
3	15-month-old, female child, weight 9 kg, and in good health before ingestion of chlordane	oral (ingestion)	Not quantified (estimated to be <100 mg or <11.1 mg/kg bw)	wettable powder (50% chlordane); 1 tablespoon chlordane diluted to 1 quart with water	single exposure	none	Generalized tremor, incoordination, ataxia, convulsions (alternating tonic and clonic phases); irregular respirations; increased pulse rate; dilated pupils; irritability; risus sardonicus; opisthotones, bilateral ankle clonus, and generalized hyperactive reflexes; mild hypochromatic anemia (not clearly associated with exposure in the literature).	Lensky and Evans, 1952

TABLE VI-1 (cont.)

Case No.	Case Description	Route of Exposure	Dose	Formulation	Duration of Exposure	Possible Confounding Factors	Symptoms	Reference
4	4-year-old, Spanish American, female child weighing 11 kg (24 pounds	oral (ingestion)	Not quantified (based on GI absorption estimated to be ≥g.62 mg or ≥0.15 mg/kg bw)	45% chlordane emulsifiable concentrate	single exposure	none	Clonic convulsions; coordination loss; hypoflexia; increased excitability; sinus tachycardia and right axis deviation, as evidenced by ECG; increased body temperature (100.3°F) on the day after exposure; absence of spontaneous vomiting.	Aldrich and Holmes, 1969
5	20-month-old, white, male child, weighing 12.7 kg (28 pounds)	oral (ingestion)	Not quantified	oil solution containing 74% technical grade chlordane	single exposure	Subject had a hypochromic microcytic anemia, which is usually associated with a history of poor iron nutrition.	Vomiting; frightened appearance; short interrupted seizures (both general and focal) accompanied by brisk deep tendon reflexes in all extremities; transient increased body temperature; hemic heart murmurs; transient increased white blood cell count; abnormal serum alkaline phosphatase and thymol turbidity levels at 3 months after exposure.	Curley and Gerrettson, 1969
6	47-year-old, married, male nurseryman; considered to be a "fairly heavy pipe (tobacco) smoker"	dermal (skin contact with soil containing chlordane)^a	Not quantified	NR	2 years	concurrent exposure to DDT, hormone sprays and arsenic pesticides	Jacksonian and grand mal convulsions; EEG revealed a generalized dysrhythmia with no localizing features; liver function tests were normal.	Barnes, 1967

TABLE VI-1 (cont.)

Case No.	Case Description	Route of Exposure	Dose	Formulation	Duration of Exposure	Possible Confounding Factors	Symptoms	Reference
7	23-year-old, woman, who had worked with chlorinated hydrocarbons (including chlordane) for 2 years preceding acute exposure	dermal	Not quantified	suspension of 25 pounds of DDT, 39 pounds of velsicol, AR 50 and 10 pounds of triton x-100	single exposure	occupational exposure to chlorinated hydrocarbons for 2 years before acute exposure, but with no symptoms of previous drug intoxication; acute exposure also included DDT, velsicol, AR 50 and triton X-100	Confusion; convulsions; at autopsy, nonspecific pathological changes were seen in the kidneys, brain and lungs.	Derbes et al., 1955
8[b]	56-year-old, male dentist in excellent health until ~5 months after initiation of exposure	dermal and inhalation	Not quantified	wettable powder; 74% technical grade chlordane diluted 1:90 with water	~3-5 hours over 2 months for dermal; ~300 hours over 5 months for inhalation; exposures began simultaneously	"occasional" use of 5 mg diazepam (valium) and 200 mg ethchlorvynol (placidyl) before and during exposure	Starting at ~5 months after initiation of exposure: tachycardia; night-sweats; fatigue; shortness of breath; pale conjunctivae; presence of S_3 gallop; occult blood in stool; persistent elevated body temperature (38.8-39.4°C) with prostration, chills, and sweat; pallor of skin and mucous membranes; cholestatic hepatitis.	Furie and Trubowitz, 1976
9	No case description in the literature	dermal	Not quantified	73.3% technical grade chlordane in petroleum distillate	NR	NR	dizziness, nausea and vomiting	Klemmer et al., 1977
10	3-month-old infant	dermal and/or inhalation	Not quantified	NR	NR	concurrent exposure to aldrin	lethargy	Klemmer et al., 1977

[a] This subject handled pipe tobacco after handling soil contaminated with chlordane, probably resulting in inhalation exposure in addition to the known dermal exposure.

[b] This case is also discussed in Table VI-2 (Case No. 17).

NR - Not reported

doses of <11.1 mg/kg by a 15-month-old infant (Case No. 3) (Lensky and Evans, 1952) and ≥0.15 mg/kg by a 4-year-old child (Case No. 4) (Aldrich and Holmes, 1969). Curley and Garretson (1969) observed CNS effects in a 20-month-old infant (Case No. 5), but the dose level was not quantified. Dose levels for exposures by the dermal and/or inhalation routes were not quantifiable (Barnes, 1967; Derbes et al., 1955; Furie and Trubowitz, 1976; Klemmer et al., 1977).

Blood Dyscrasias Associated with Exposure to Chlordane and Heptachlor. Exposure to chlordane and heptachlor by skin contact and/or inhalation has been associated with several blood dyscrasias, including four cases of aplastic anemia (Infante et al., 1978; Klemmer et al., 1977), one case of refractory megaloblastic anemia (Furie and Trubowitz, 1976), one case of acute stem cell leukemia (Infante et al., 1978), one case of acute lymphoblastic leukemia (Infante et al., 1978) and one case of acute myelomonocytic leukemia (Infante et al., 1978). Exposures were a result of indoor or outdoor applications or a combination of the two. These clinical case studies are summarized in Table VI-2.

Three cases (Nos. 1, 2 and 5) were mixed exposure with other pesticides, which may also be associated with blood dyscrasias in humans.

In addition to the clinical case studies described in Table VI-2, Muirhead et al. (1959) reported one case of hemolytic anemia associated with exposure (route of exposure not specified) to chlordane, heptachlor, dieldrin and toxaphene, and five cases of aplastic anemia, of which two were associated with exposure (routes of exposure not specified) to chlordane and

TABLE VI-2

Summary of Cases of Blood Dyscrasias Associated with Exposure to Chlordane or Heptachlor, Either Alone or with Other Agents

Case No.	Case Description	Description of Exposure	Exposure to Other Agents	Type of Blood Dyscrasia	Reference
11	male, 15 years old	Dermal by contact with outdoor spray for 5 months and inhalation by indoor application of chlordane.	Isotox (containing carbaryl)	aplastic anemia	Infante et al., 1978
12	male, 28 year old, self-employed realtor	Extensive use of a 75% chlordane formulation for 6 months, before onset of symptoms (exposure not otherwise specified).	diazinon, various paints, thinners	aplastic anemia	Infante et al., 1978
13	male, 68 years old	Use of chlordane outdoors and indoors (74% chlordane) more than once during the 3 years preceding the onset of symptoms.	undetermined	aplastic anemia	Infante et al., 1978
14	female, 9 years old	Exposure to chlordane applied to the interior of her home initially at the age of ~6 months and annually thereafter.	none	acute stem cell leukemia	Infante et al., 1978
15	male, 23 years old, employed by a lawn care firm	Spraying lawns with chlordane and other pesticides for 3 years before onset of symptoms.	Banvel D, diazinon, Bursban, 2,4-D, paints, strippers, thinners	acute lymphoblastic leukemia	Infante et al., 1978
16	male, 37 years old	Use of a 44% chlordane formulation for 10 years before onset of symptoms, "frequent" use indoors of full strength (application with a paint brush) and several times/year outdoors of diluted chlordane.	none	acute myelomonocytic leukemia	Infante et al., 1978

TABLE VI-2 (cont.)

Case No.	Case Description	Description of Exposure	Exposure to Other Agents	Type of Blood Dyscrasia	Reference
17*	male, 56 years old, dentist	Exposure to 74% technical grade chlordane diluted 1:90 with water for ~3-5 hours skin contact over 2 months and ~300 hours by inhalation over 5 months; exposures began simultaneously.	"Occasional" use of 5 mg diazepam (valium) and 200 mg ethchlorvynol (placidyl) before and during exposure	refractory megaloblastic anemia	Furie and Trubowitz, 1976
18	engineer; no other details provided	Treated an area under a house with 250 gallons of 4.5% chlordane emulsion.	not specified	aplastic anemia	Klemmer et al., 1977

*This case is also discussed in Table VI-1 (Case No. 8).

heptachlor only and three to chlordane, heptachlor and unspecified other drugs. Also, Loge (1965) reported 12 cases of aplastic anemia with pancytopenia, of which three were associated with chlordane exposure only and nine in conjunction with other drugs, and one case of thrombocytopenia and two cases of leukopenia agranulocytosis associated with exposure to chlordane in conjunction with other drugs.

The American Medical Association, Council on Drugs, as reported in the Registry on Blood Dyscrasias, concluded that there is evidence that a "specific cause-effect relationship exists" between exposure to chlordane and resulting blood dyscrasias, particularly pancytopenia, thrombocytopenia, leukopenia and anemia (Anonymous, 1962).

Neuroblastoma Associated with Pre- and/or Postnatal Exposure to Chlordane and Heptachlor. Infante et al. (1978) described five cases of neuroblastoma in children with a pre- and/or postnatal history of exposure to technical grade chlordane containing ~38.5% chlordane and 3-7% heptachlor. These cases are summarized in Table VI-3. Nine additional cases of neuroblastoma were diagnosed during the same time period (December 1974 to February 1976) at the same pediatric hospital, but pre- and/or postnatal exposure to chlordane and heptachlor were not ascertained. That effects may be induced by prenatal exposure is consistent with the documented transplacental transfer of chlordane in humans (Curley et al., 1969; Wasserman et al., 1972, 1974; Zavon et al., 1969).

TABLE VI-3

Cases of Neuroblastoma Associated with Exposure to Chlordane and Heptachlor*

Case No.	Case Description	Description of Exposure and Confounding Factors	Pathological Condition
19	female, age 2 years and 8 months	Technical grade chlordane was applied to both the interior and exterior of the 28-year-old mother's home during the first trimester of gestation. Application to the basement, where the mother spent 25-30 hours/week typing, included sealing chlordane in holes drilled in cement blocks. The house was not well ventilated, because of cold weather. The mother took two valium tablets and received general anesthesia (thiopental sodium, halothane and nitrous oxide) early in the pregnancy. The exterior of the house was sprayed with chlordane a second time one year later.	**Neuroblastoma (Stage III)**
20	male, age 4 years and 5 months	Technical grade chlordane was applied to both the interior and exterior of the home every 6 months from one year before conception. The mother took aspirin during pregnancy.	**Neuroblastoma (Stage IV)**

TABLE VI-3 (cont.)

Case No.	Case Description	Description of Exposure and Confounding Factors	Pathological Condition
21	female, age 4 years and 4 months	Technical grade chlordane was applied to the interior of the home when the girl was 23 months old.	Neuroblastoma (Stage IV)
22	male, age 3 years and 9 months	Technical grade chlordane was applied to both the interior and exterior of the home 2 years before the child was born and when he was 2 years old. Between the two applications of chlordane, application of a commercial pesticide was intermittent.	Neuroblastoma (Stage IV)
23	female, age 6 years and 5 months	Chlordane dust was applied to the exterior foundation of the house when the child was 3 years and 8 months old (autumn of the year) and again in the spring of the next year.	Neuroblastoma (Stage IV)

*Source: Infante et al., 1978

Epidemiological Studies

Wang and MacMahon (1979a) conducted a retrospective cohort mortality study of 16,126 professional pesticide applicators that was undertaken to study the mortality patterns of these workers. The cohort was selected from three pest control companies with offices in over 40 states. Personnel records for all persons in job categories potentially involving exposure to various pesticides and employed between January 1, 1967 and June 30, 1976, were reviewed for two of the companies. For the third company, employee records were not available for the period prior to January 1, 1968; therefore, this date was used as the earliest date of eligibility for this company, with a closing date of December 31, 1976. From 44,083 records, 16,126 subjects were identified who met the following criteria: 1) male; 2) employed for 3 months or more; and 3) social security number, date of birth, and employment dates were also available. Of those not eligible, a sample of 4000 were examined; 11% had been excluded because they were female, 71% because they were employed for <3 months, and 18% because of missing identifying information.

Individual follow-up was not attempted. Identifying information for the 16,126 eligible subjects was submitted to the Social Security Administration (SSA). The SSA identified 311 deaths, of which 269 death certificates were obtained from the states or registration areas. The authors stated that because there was no reason to believe that the 42 deaths for which death certificates could not be found were distributed differently than those for which certificates were found, observed numbers were "corrected" by distributing the 42 cases according to the distribution of obtained death certificates and adding the results to the observed values for those with obtained death certificates.

In order to develop some measurement for intensity of exposure to pesticides, job titles were classified as either normal, moderate or heavy exposure. Particular interest was focused on those applicators exposed to chlordane and heptachlor by conducting separate analyses for persons ever holding jobs as termite control operators, a job category likely to have exposure to these two pesticides. If a worker held more than one job he was classified under the category of exposure corresponding to the job held for the longest period of time.

Expected numbers of deaths by cause were calculated by means of a computer program by Munson (1974), using national mortality rates of white males specific for 5-year categories of age and calendar year. SMRs were calculated along with 95% confidence intervals by an extension of the Cornfield method. The authors presented SMRs based on the corrected number of observed deaths. For this review, SMRs based on the true numbers of observed deaths were calculated whenever possible. Based on the corrected observed numbers, the SMR for all causes was 84 (observed=311, 95% CI 75-94). The SMR for all malignant neoplasms was 83, which was statistically nonsignificant. Increased SMRs were observed for skin cancer (SMR=173, statistically nonsignificant) and for bladder cancer (SMR=277, corrected observed=3.5, 95% CI 101-761). Statistically significant SMR deficits were observed for malignant neoplasms of the digestive organs and peritoneum (SMR=46, corrected observed=6.9, 95% CI 22-95), respiratory system diseases

(SMR=29, corrected observed=4.6, 95% CI 12-70), diseases of the digestive system (SMR=55, corrected observed=11.6, 95% CI 31-98), and all other causes (SMR=355, corrected observed=15, 95% CI 33-90).

SMRs for various specific causes of death using the true number of observed deaths can be calculated and are presented here. For the total cohort they are as follows: SMR for all causes of death is 84 (observed=269); for malignant neoplasms, the SMR is 75 (observed=47), for cancer of the digestive organs and peritoneum, the SMR is 40 (observed=6); for skin cancer, the SMR is 150 (observed=3); for bladder cancer, the SMR is 231 (observed=3); for nonmalignant diseases of the respiratory system, the SMR is 25 (observed=4), and for nonmalignant diseases of the digestive system, the SMR is 48 (observed=10).

Analyses were also conducted for those workers classified as termite control operators, since they were thought to have had the most exposure to chlordane and heptachlor. SMRs were presented for termite control operators and for all other applicators for various causes of death. These SMRs were based on corrected observed values. The SMR for all causes of death for the termite control operators was 92 and for all other applicators was 78. For malignant neoplasms, the SMR for both groups was 83. Elevated SMRs were observed for each group for cancer of the skin (SMR for termite control operators=148, SMR for all other applicators=187), and cancer of the bladder (SMR for termite control operators=215, SMR for all other applicators=187). None of the above-mentioned SMRs was statistically significant. The authors stated that the only significant SMRs ($p<0.05$) observed for termite control operators were for cancer of the digestive organs (SMR=20, corrected observed=1.2) and for cerebrovascular disease (SMR=39, corrected observed=2.4). The authors also stated that there were no significant findings in the "all other applicator" group, nor were there significant differences in SMRs between the two groups. (The method used to determine significant differences between the two groups was not reported.) A nonsignificant increase in lung cancer was restricted to the "all other applicator" group (SMR=131, corrected observed=16.9). It is possible to calculate SMRs using the true number of observed deaths for cancer of the skin and bladder. For skin cancer, the SMR for termite control operators was 120, and for all other applicators was 167. For bladder cancer, the SMR for termite control operators was 200, and for all other applicators was 250.

Workers were classified according to intensity of exposure into three groups: minimal, intermediate and highest exposure. None of the causes of death exhibited an increase in SMR with an increase in intensity of exposure. In fact, SMRs based on corrected observed values for lung cancer and skin cancer tended to decrease with intensity of exposure. For lung cancer SMRs of 138, 120 and 87 were observed for minimal, intermediate and highest exposure, respectively, and for skin cancer SMRs of 198, 187 and 138 were observed, respectively. The authors did not discuss the statistical significance of these findings.

A latency analysis was performed for lung cancer. However, few workers were followed for >10 years since first employment, and none of the lung cancer deaths had been followed for that length of time. Positive trends toward an increase in lung cancer deaths occurred as the period of latency increased.

There are several limitations to this study. The major limitation is the distribution of deaths without death certificates according to the distribution of those with death certificates. If the age distribution (or the distribution of any other disease-related variable) of the group without death certificates had been different from that of the group with death certificates, the cause-specific death distribution could have been different, and could have resulted in inaccurate calculation of observed numbers.

There was also no quantitative information available on levels of exposure, and duration of exposure could only be assessed for lung cancer because of small numbers of observed cases. It is not possible to assess risks for chlordane and heptachlor exposure independently of the risks from other possible exposures. While the termite control applicators may have had a greater likelihood of chlordane and heptachlor exposure than other applicators, their exposure to other pesticides cannot be ignored.

The authors did not individually follow up each cohort member. The only method used to ascertain vital status was a search through SSA records. Thus, the number of deaths may have been underreported. There was no control of confounding factors such as smoking and alcohol consumption. This study provides inadequate evidence on the carcinogenicity of chlordane and heptachlor.

Wang and MacMahon (1979b) conducted a retrospective cohort mortality study that was undertaken in two chlordane and heptachlor manufacturing plants between 1946 and 1976. Personnel records were available for 951 workers who had ever worked at a chlordane production plant in Marshall, IL, and for 1425 workers who had ever worked at a heptachlor and endrin plant in Memphis, TN before the spring of 1976. Of these, 1403 subjects were identified who met the following criteria: 1) male; 2) who had worked for >3 months; and 3) "adequate" identifying information was available. Of the 973 excluded, 7% were excluded because they were female, 64% because they worked <3 months, and 29% because identifying information was not available.

The SSA identified 104 deaths in the study cohort through the end of 1975. Nine additional deaths, not identified by SSA, were discovered during the conduct of a separate study (reference not reported) that individually followed up terminated workers from these plants. Death certificates were obtained from the appropriate states for the 113 deaths (98% of those ascertained) and were coded according to the 8th revision of the ICD by one of the authors (Wang). Cause-specific SMRs were calculated by means of Munson's (1974) computer program, using national mortality rates for white males by age and calendar year in 5-year groups. For the computation of person-years, the beginning dates were January 1, 1946 for the Marshall plant and January 1, 1952 for the Memphis plant, or at the end of 3 months of employment if that date was later than the appropriate date above. The authors calculated 95 percent confidence intervals by an iterative method based on mid-p values.

The authors attempted to correlate the intensity of exposure with mortality experience. Complete occupational histories were not available for each worker, and serum levels of pesticides actively used in 1975 and 1976 did not correlate with a classification of presumed exposure based on job category; however, no data were presented. Thirty-four percent of the study cohort had <10 years duration of follow-up, 25% had 20-29 years of follow-up, and 11% had >29 years of follow-up.

The overall SMR for all causes of death was 72 (observed=113, 95% CI 59-86), confirming the healthy worker effect. A deficit was seen in the SMR for all cancers (SMR=82), but this was not statistically significant. Statistically nonsignificant deficits were observed for malignant neoplasms: for cancer of the digestive organs and peritoneum, the SMR was 82; for lymphatic and hematopoietic cancers the SMR was 30; and for all other cancers combined the SMR was 45. A statistically nonsignificant excess was observed for lung cancer; the SMR was 134 ($p>0.05$). The only significantly elevated SMR was for cerebrovascular disease (SMR=183; observed=17, 95% CI 110-287). Only one death was attributed to liver cancer: an 81-year-old man who died in 1958 and had worked for the company for 5 years beginning in 1944. A significant deficit was observed for ischemic heart disease (SMR=69, observed=37, 95% CI 49-94).

Because the SMR for lung cancer was elevated (SMR=134), though not statistically significant, the distribution of lung cancer deaths across other variables was investigated. For workers <35 years old at entry into occupation and <50 years old at observation, the authors noted that the difference between observed and expected (5 and 1.2, respectively) was statistically significant ($p<0.01$). The relationship between duration of employment and duration of follow-up or latency for lung cancer was studied. The numbers were small and no overall pattern was observed. However, the results indicate that there was a statistically significant deficiency (p-value not reported) in lung cancer deaths (observed=1, expected=4) among those exposed for 20 or more years. Conversely, among those employed for 10-19 years and followed for 10-19 years, there was an excess risk (observed=6, expected=2.1).

There are limitations to this study. There was no information available on levels of exposure or duration of exposure. It is not possible to assess risks for chlordane and heptachlor exposure independently of the risks of endrin exposure, a pesticide also manufactured at the Memphis plant.

The cohort included all plant employees, including those with little potential for exposure, such as office workers; and therefore, the risk of cancer may have been underestimated for those directly involved in chlordane and heptachlor manufacture. While the period of follow-up was long, the size of the cohort was very small. Thus, the study had very little power to detect a real difference if one was present.

The authors did not individually follow up each cohort member. Therefore, the number of deaths may have been underreported. Excluded from the cohort were 282 individuals with missing data, representing ~29% of the final cohort population. The authors provide no data with which to assess the impact of excluding these workers from the cohort; however, this may be a random occurrence. If this assumption is true, then their exclusion poses no bias to the estimates of risk.

There was no control of confounding variables such as smoking. However, a significantly low SMR was observed for ischemic heart disease, a disease for which smoking is a well-documented risk factor. The SMR for other respiratory diseases was also low, though not significantly. This result suggests that the cohort in question smoked at levels below that of males nationally, and provides additional evidence that the reported excess incidence of lung cancer (SMR=134) may have been an occupationally related increase. Finally, race was assumed to be white for all study subjects. As no deaths among nonwhites were observed, this assumption may be a valid one. Thus, this study provides inadequate evidence for the carcinogenicity of chlordane and heptachlor.

Ditraglia et al. (1981) conducted a retrospective cohort mortality study of employees at four organochlorine pesticide manufacturing plants. All workers (race and sex not specified) who had at least 6 months of employment in pesticide manufacture prior to December 31, 1964 were included in the study population. Vital status was determined for each worker as of December 31, 1976, through the SSA, State motor vehicle offices, U.S. Postal Mail Corrective Services, and "other" sources.

Four separate cohorts representing four pesticide plants comprised the study population. Only one plant had manufactured chlordane and had done so since 1946. No other pesticides were manufactured at this plant, but other chemical products manufactured there included chlorine and dicyclopentadiene. The cohort at plant 1 consisted of 327 individuals, representing 8354 person-years of observation. Three percent were lost to follow-up. Plant 2 had manufactured heptachlor since 1951. Endrin was also produced at this plant in addition to chlorine, chlorendic anhydride, hexachlorocyclopentadiene and vinyl chloride. The cohort at this plant consisted of 305 workers (5672 person-years of observation), and 5% were lost to follow-up. The two remaining plants were not involved in the production of chlordane or heptachlor and, thus, results are not discussed here for these two plants. The chlordane and heptachlor plants had previously been studied by Wang and MacMahon (1979b), who combined the two plant populations into a single cohort with a longer period of follow-up in comparison to the follow-up period reported in this study (Ditraglia et al., 1981).

Death certificates for all known decedents were obtained and coded by a nosologist to the ICD-A in effect at the time of death. Those with an unknown vital status were assured alive as of December 31, 1976. SMRs were calculated by using the U.S. white male age-, calendar time- and cause-specific mortality rates. Statistical significances between the observed and expected values were tested with the Poisson distribution. Confidence intervals were presented for the SMR estimates, but the method of calculation was not reported by the authors.

Statistically significant (p<0.05) deficits were observed for all causes of death at the chlordane plant (SMR=68, observed=59, 95% CI 52-87) and at the heptachlor plant (SMR=66, observed=24, 95% CI 42-98). For deaths because of malignant neoplasms, the chlordane plant had an SMR of 69, and the heptachlor plant had an SMR of 91, none being statistically significant. At the chlordane plant, risks were elevated for stomach cancer (SMR=303), rectal cancer (SMR=178), pancreatic cancer (SMR=110), and respiratory system cancer (SMR=110). None of these risks was statistically significant. At the heptachlor plant, excess risk was observed for intestinal cancer (SMR=175), respiratory system cancer (SMR=122), and cancer of the bladder and urinary system (SMR=606.). These risks were also not statistically significant.

An analysis by latency was also carried out. SMRs were calculated for deaths due to all malignant neoplasms according to latency, which was defined as the number of years from date of first employment. Latency was categorized into three time periods: 1) <10 years since first employed, 2) 10-19 years since first employed, and 3) 20 or more years since first employed. In the chlordane plant, SMRs of 66, 90 and 60 were reported for all malignant neoplasms for the three latency time periods, respectively. The individual point estimates were not significant. There were no observed deaths in the <10-year latency period in the heptachlor plant. The SMR for all malignant neoplasms for the 10- to 19-year latency period was 91, and for the 20 or more year period the SMR was 162. These were not statistically significant; however, there may have been a positive trend of increasing risk with increasing latency at the heptachlor plant.

This study revealed excess risk of cancer at various tissue sites for all workers at these two plants. While none of the SMRs was statistically significant, the SMR of 303 (observed=3) for stomach cancer in the chlordane plant may be important. However, there are limitations to this study.

No information on quantitative exposures were provided. It was not possible to assess the effects of chlordane or heptachlor independently of the other toxic chemicals at the two plants, some of which are known to be carcinogenic. No attempt was made to exclude or adjust for the effects of sex or race, nor was there an effort to control for other confounding variables such as smoking or alcohol consumption. However, the size of the study populations would preclude any real analysis of these variables. Workers with little or no occupational exposure to these chemicals (i.e., office workers, etc.) were included in the cohort. Thus, risks may have been underestimated for workers involved in the day-to-day manufacture of chlordane or heptachlor. The size of the study population was small in spite of follow-up periods of 25 years or more. Thus, the power of this study to detect a statistically significant result is limited. This study provides inadequate evidence to link chlordane or heptachlor exposure to cancer.

High Risk Subpopulations

There are no clear identifiable high risk subpopulations that are inherently susceptible to exposure to chlordane, heptachlor or heptachlor epoxide. Various blood dyscrasias have been associated with exposure to chlordane and heptachlor (Infante et al., 1978; Furie and Trubowitz, 1976; Klemmer et al., 1977). Because of the large number of agents associated with these blood dyscrasias and the small incidence of these conditions, Infante et al. (1978) hypothesized that an idiosyncratic mechanism for susceptible individuals may be involved, but that identification of members of this sensitive subpopulation would be virtually impossible.

Several groups may be more affected than the general population because of high exposure rather than intrinsic susceptiblity to chlordane, heptachlor or heptachlor epoxide. These high exposure groups include exterminators; agricultural applicators; workers employed in the manufacture of chemicals; infants nursing on human milk because of maternal excretion of heptachlor epoxide and oxychlordane, the major mammalian metabolites of heptachlor and chlordane, respectively, in mothers' milk (Savage et al., 1973; Strassman and Kutz, 1977); the developing fetuses of women exposed to chlordane because of transplacental transfer (Curley et al., 1969; Wasserman et al., 1972, 1974; Zavon et al., 1969); consumers of fish and shellfish (especially freshwater); persons residing in houses after termite extermination treatment with chlordane and heptachlor.

Summary

DeLong and Ludwig (1954) did not observe treatment-related effects in a human exposed to 7% chlordane vapors for 15 minutes every 3 days for 12 weeks. The clinical case studies of acute exposure to chlordane and heptachlor document a pattern of CNS effects similar to that found in animals. The course of poisoning frequently includes irritability, salivation, labored respiration, muscle tremors, convulsions and death with or without an immediately preceding period of deep depression. These symptoms were seen at doses as low as 32 mg/kg bw (estimated to be 10 mg/kg bw after vomiting) in an 18-year-old female (Dadey and Kammer, 1953) and 104 mg/kg bw in a 32-year-old female (Derbes et al., 1955). Several blood dyscrasias, including four cases of aplastic anemia (Infante et al., 1978; Klemmer et al., 1977), one case of refractory megaloblastic anemia (Furie and Trubowitz, 1976), one case of acute stem cell leukemia (Infante et al., 1978), one case of acute lymphoblastic leukemia (Infante et al., 1978) and one case of acute myelomonocytic leukemia (Infante et al., 1978), have been associated with exposure to chlordane and heptachlor. Infante et al. (1978) also suggested an association with pre- and/or postnatal exposure to chlordane and heptachlor.

Three epidemiological studies of workers exposed to chlordane and heptachlor have been reported. One of these studies, conducted in chlordane/heptachlor applicators, was considered inadequate in sample size and in duration even though it showed increased morality from bladder cancer (SMR=277, $p<0.05$). A second study showed an increased mortality from lung cancer (SMR=134), but the increase was not statistically significant. The mortality from cerebrovascular disease was statistically significant (SMR=183, $p\leq 0.05$). Of the 1043 men involved in the study, only one liver cancer was reported. The third study involved 2141 workers exposed to organochlorine pesticides. One of the four plants involved in pesticide manufacture produced chlordane and one produced heptachlor. The SMR for malignant neoplasms was 69 at the chlordane plant and 91 at the heptachlor plant. There was an excess risk for cancer in various tissues; none was statistically significant. The last two studies were carried out in chlordane/heptachlor manufacturing plants.

All of these studies have several limitations. Neither the quantitative nor length of exposure histories are available for chlordane/heptachlor for the populations studied. They were also exposed to other pesticides and chemicals. Adjustments for these other chemical exposures and other confounding factors, like smoking and alcohol consumption, were not considered in any of these studies. All of the study populations were small. In the pesticide applicator study, individual follow-up was not undertaken and the data were missing on 10.3% of the decedents reported by the Social Security Administration.

Because of these methodological limitations and the limited data, it is difficult to establish either a negative or positive association between chlordane/heptachlor and carcinogenicity. Hence, these studies are considered inadequate epidemiologic evidence.

VII. MECHANISMS OF TOXICITY

Mechanism of Neurotoxicity

As discussed in the Effects of Acute Exposure and the Effects of Subchronic and Chronic Exposures Sections in Chapter V, one of the most commonly observed effects of chlordane or heptachlor exposure was stimulation of the CNS. Several investigators have studied the mechanism for neurotoxicity. Hyde and Falkenberg (1976) studied the effect of chlordane on brain potentials in male Sprague-Dawley rats that were surgically implanted with electrodes for EEG monitoring. Chlordane in a Tween-80-saline vehicle was administered intraperitoneally at 0.15, 1.75 or 25.0 mg/kg bw/day for 48 days. Control rats received vehicle only. Dose-related changes in neuroelectrical activity (frequency, amplitude and waveform changes) occurred during exposure, with increasing incidence as the duration of exposure progressed. No outward signs of toxicity were observed. The EEG changes included a shift toward fast beta rhythms (>25 Hz), elevated amplitudes, reductions of delta (0.5-3 Hz) and theta (4-7 Hz) frequencies. Sinusoidal waves were replaced by sharp, complex discharges. During the last 6 days of chlordane injection, the rats were starved and EEGs monitored. Abnormally high voltage potentials that suggested lethal patterns were observed. It was postulated that food deprivation resulted in mobilization of fat depots of chlordane and/or metabolites, which then became neuroconcentrated. These EEG disturbances did not return to normal when chlordane treatment was discontinued, suggesting the persistence of chlordane as a neurotoxin.

St. Omer and Ecobichon (1971) studied the time course of changes in acetylcholine content of rat brain with respect to the time course of toxic symptoms induced by heptachlor. Female Wistar rats with carotid artery cannulation were injected with a single dose of 13.0 mg/kg bw of reference grade heptachlor in a peanut oil-lecithin emulsion. Vehicle injected rats served as controls. Groups of six rats were sacrificed at 5, 15, 25 and 30 minutes after injection and brain extracts were prepared for acetylcholine content determination. Animals not sacrificed after treatment had mild tremors progressing to severe tremors in 2-26 minutes. Within 10-20 minutes after treatment, there were episodes of running, rolling and leg-paddling, followed by mild tonic-clonic seizures and inactivity at 18-26 minutes. Death of chlordane-treated rats occurred within 2 hours. The average acetylcholine content of rat brain extracts was ~6.5 μg/g wet tissue at 5 minutes (vehicle control value = 3.3 μg/g and remained constant) and ~10.5 μg/g at 15 minutes. Acetylcholine levels declined to ~9.5 μg/g at 25 minutes and ~9.0 μg/g at 30 minutes after injection, but remained elevated above control levels.

In similarly conducted experiments, St. Omer (1971) studied the time course of changes in ammonia and glutamine content of rat brain. The time course of toxic symptoms was as previously described (St. Omer and Ecobichon, 1971). The ammonia content of the brain increased significantly to 2-fold above vehicle control levels at 25 minutes after treatment ($p<0.05$). The glutamine content was unaffected. The increased ammonia level coincided temporally with mild seizures in heptachlor treated rats. It was noted that the level of ammonium ions in the brain increase during states of CNS excitation. Ammonia in the brain is removed by conversion to glutamine. Since, following heptachlor treatment, it appeared that this conversion did not take place, the accumulation of ammonia without subsequent removal induced the neurotoxicity. St. Omer (1971) concluded that heptachlor acted by a mechanism that interfered with the production or utilization of ammonia.

The biochemical mechanism of neurotoxicity of heptachlor epoxide was studied by Yamaguchi et al. (1979) with respect to inhibition of synaptic ATPases. Synaptosomal, microsomal, synaptic vesicle and synaptic membrane fractions were prepared from homogenates of brain from Sprague-Dawley rats. The sensitivities of various ATPases in these fractions to heptachlor epoxide in vitro, in decreasing order, were as follows: Ca^{2+}, Mg^{2+}-ATPase (66.8-76.9% inhibition) > Mg^{2+}-ATPase (31.2-48.9% inhibition) > Ca^{2+}-ATPase (26.5-48% inhibition) > Na^{+}, K^{+}-ATPase (0-18.4% inhibition). The inhibition of Ca^{2+}, Mg^{2+}-ATPase was greatest in the synaptic vesicle fraction (which contained synaptic membrane components). The inhibition of Ca^{2+}, Mg^{2+}-ATPase by heptachlor epoxide was shown to reduce the binding capacity of Ca^{2+}. It was suggested that the resulting increased level of Ca^{2+} in the presynaptic region promoted the release of neurotransmitter (e.g., acetylcholine) contained in the synaptic vesicles, which resulted in the appearance of toxic symptoms.

This phenomenon was further studied (Yamaguchi et al., 1980) using rat brain synaptosomes and glutamate as the model excitatory neurotransmitter. The release of transmitter is controlled by synaptic vesicle concentration of Ca^{2+}. In a series of experiments, it was demonstrated that heptachlor epoxide stimulated the uptake and inhibited the release of Ca^{2+} by synaptosomes, leading to accumulation of Ca^{2+}. Heptachlor epoxide also reduced the uptake of Ca^{2+} by the endoplasmic reticulum. Thus, Ca^{2+} within the synaptosomes was more available to synaptic vesicles to trigger release of transmitter. The sequestration of Ca^{2+} within the synaptosome was related to the observed inhibition of Ca^{2+}, Mg^{2+}-ATPase (Yamaguchi et al., 1979). To confirm the in vitro results, heptachlor epoxide was administered intraperitoneally to rats at 200 mg/kg bw in corn oil. Violent convulsions occurred within 5 hours, at which time the rats were killed and synaptosomes prepared. The Ca^{2+}, Mg^{2+}-ATPase within synaptosomes was inhibited and a 4-fold greater concentration of heptachlor epoxide was found in synaptosomes than in whole brain.

Folmar (1978) found that in vitro rat brain microsomal N^+, K^+-ATPase was significantly inhibited by chlordane (82%) and heptachlor (72%), suggesting a role of this ATPase in the neurotoxicity of these insecticides.

Mechanisms of Effects on Endocrine and Reproductive Organs

In an attempt to explain the action of chlordane and heptachlor on ventral prostate homeostasis (e.g., increased androgen receptor site content, decreased protein content) observed in rats following subchronic pesticide feeding (see Effects of Subchronic and Chronic Exposures Section in Chapter V), Shain et al. (1977) studied 5α-dihydrotestosterone binding to androgen receptors in vitro. Chlordane and heptachlor were found to be poor inhibitors of 5α-dihydrotestosterone binding to the receptor of ventral prostate cytoplasmic extracts or tissue mince preparations when compared with the inhibition by parathion. It was concluded that 5α-dihydrotestosterone binding inhibition was not a good predictor of the pesticide effect on ventral prostate homeostasis. An alternative mechanism was not presented.

Welch et al. (1971) found that chlordane administered intraperitoneally to mice at a dose of 25 mg/kg bw once a week for 3 weeks resulted in decreased fertility (see Teratogenicity and Other Reproductive Effects Section in Chapter V). A possible mechanism was investigated. Microsomes prepared from ovariectomized mice that had been pretreated with chlordane increased the metabolism of estrogen to polar metabolites by 3-5 times the control level. In addition, the in vivo uterotropic action of exogenous estrogen was 88-98% inhibited in mice pretreated with chlordane. Similar results were obtained in the in vivo and in vitro experiments conducted in rats with chlordane (cis- and trans-isomers) and heptachlor.

Enzyme Induction and Related Mechanisms

Karel and Saxena (1976) observed increased levels of blood glucose, serum protein, serum alkaline and serum acid phosphatases in gerbils that had been treated intramuscularly with chlordane (see the Effects of Acute Exposure and the Effects of Subchronic and Chronic Exposures Sections in Chapter V). These investigators speculated that the increased level of serum protein was a result of increased incorporation of amino acids into protein in the liver, with subsequent release into the blood stream. The increased level of serum alkaline phosphatase may have resulted in part from increased activity of osteoblasts and in part by release from necrotic liver cells. Release of acid phosphatase from degenerating liver cells would result in higher serum levels of this enzyme, as well. The increase in blood glucose was explained as follows. Chlordane treatment activated the sympathetic nervous system, resulting in adrenal medulla secretion of adrenaline, and this stimulated anterior pituitary secretion of ACTH. ACTH activity resulted in production of glucocorticoid hormones, which in turn enhanced gluconeogenesis.

Enhanced gluconeogenesis was also observed in liver and kidney cortex of rats following acute or subchronic exposure to chlordane, heptachlor and heptachlor epoxide (Kacew and Singhal, 1973; Kacew et al., 1973; Singhal and Kacew, 1976). The enhancement was evidenced by increased activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase, as well as increased levels of blood glucose, serum urea and decreased liver glycogen (see the Effects of Acute Exposure and the Effects of Subchronic and Chronic Exposures Sections in Chapter V for dose, routes, durations, etc.). These investigators found that treatment of rats with these pesticides also resulted in significantly elevated activities of adenyl cyclase and levels of cyclic AMP in the liver and kidney cortex, suggesting that synthesis of cyclic AMP was enhanced (Kacew and Singhal, 1973). Treatment of rats intraperitoneally with cyclic AMP (2x100 mg/kg bw) resulted in significantly increased activities of the four gluconeogenic enzymes, increased blood glucose and serum urea levels, and decreased liver glycogen content. Cyclic AMP, therefore, mimicked the action of pesticides (Singhal and Kacew, 1976). A mechanism of enhanced gluconeogenesis was proposed whereby the insecticides react with an adenyl cyclase receptor on the plasma membrane, thus stimulating the activity of the enzyme to synthesize cyclic AMP from ATP. Cyclic AMP, in turn, may bind with a protein kinase receptor, resulting in a cyclic AMP independent protein kinase that is translocated to the nucleus. The protein kinase then catalyzes the phosphorylation of nuclear proteins, which initiates transcription, resulting in _de novo_ synthesis of messenger RNA that induces the synthesis of gluconeogenic enzymes.

Increased activities of microsomal enzymes (phosphorothioate detoxication, O-demethylase and N-demethylase) were observed in rats fed chlordane, heptachlor or heptachlor epoxide for 13 weeks (Kinoshita and Kempf, 1970).

Effects on Cellular Respiration

Settlemire et al. (1974) reported that heptachlor stimulated the ATPase of mouse liver mitochondria, but did not stimulate mitochondrial oxidation of succinate (respiration) in the absence of ADP. Respiration was stimulated in the presence of ADP. Heptachlor had no effect on ATP synthesis in the presence of ADP. It was stated that these effects were contrary to those observed for uncouplers of oxidative phosphorylation, which are known to stimulate respiration in the absence of ADP or inorganic phosphate and prevent ATP synthesis. An explanation to account for the enhanced respiration with ADP present, that there was an "increased influx of ADP into the mitochondria," was rejected on the basis that uptake of ^{14}C-ADP did not occur. It was suggested that, on the basis of a concentration-related increase in the light-scattering response of mitochondria by heptachlor, the insecticide dramatically altered the mitochondrial membrane, resulting in the observed increase in respiration in the presence of ADP. Two possible mechanisms were proposed: increased membrane permeability to succinate or confirmational changes that increased the activity of the respiratory chain.

The effects of chlordane, heptachlor and heptachlor epoxide on beef heart mitochondrial electron transport was studied by Pardini et al. (1971). Chlordane and heptachlor inhibited the succinoxidase system to 21.2 and 5.8%, respectively, of control values. Heptachlor epoxide was not inhibitory. The activity of mitochondrial NADH-oxidase was inhibited 5.8 and

10.3%, respectively, of control values. When TMPD, which when reduced by NADH non-enzymatically donates electrons to the electron transport chain at the step between cytochrome b and cytochrome c, was added, electron transport proceeded, despite the oxidase inhibition. These results indicated that chlordane and heptachlor inhibition of electron transport occurred "on the substrate side of cytochrome c," since the inhibition of NADH oxidase was bypassed by the addition of TMPD.

Synergism/Antagonism

Harbison (1975) pretreated neonatal Sprague-Dawley rats with intraperitoneal injections of phenobarbital before intraperitoneal injection of chlordane or heptachlor. The LD_{50} of heptachlor in neonates not pretreated with phenobarbital was 531 mg/kg bw. Phenobarbital pretreatment reduced the LD_{50} to 133 mg/kg bw. The chlordane LD_{50} of 1121 mg/kg bw was reduced to 539 mg/kg bw. Since phenobarbital is a known inducer of microsomal enzymes, the enhanced toxicity may have resulted from an increased capacity of the neonate to biotransform these insecticides to more toxic metabolites.

Sperling and Ewinike (1969) reported that pretreatment of adult male rats with an oral dose of 1.8 g turpentine/kg bw/day for 3 days reduced the oral LD_{50} of heptachlor from 112 to 70 mg/kg bw. The toxicity of heptachlor epoxide was not affected.

Microsomes prepared from rats pretreated with chlordane intraperitoneally with 25 mg/kg bw/day for 3 days and challenged with an intraperitoneal injection of CCl_4 (0.5 mℓ of 25% solution) had elevated levels of NADPH-cytochrome c reductase when compared with non-pretreated controls, although the increase was not statistically significant (Stenger et al., 1975). The cytochrome P-450 levels were significantly depressed by CCl_4 challenge from prechallenge values. Hepatocellular necrosis was extensive followed by CCl_4 challenge in chlordane pretreated rats. Rats treated only with CCl_4 had less extensive liver damage. Thus, chlordane pretreatment potentiated the toxicity of CCl_4, presumably by inducing microsomal enzymes that metabolize CCl_4. That chlordane induced the microsomal enzymes was demonstrated by reduced zoxazolamine paralysis time _in vivo_ and increased NADPH-cytochrome c reductase and cytochrome P-450 levels in microsomes from non-challenged rats.

Boyd and Taylor (1969) maintained 133 male weanling Wistar rats on a low protein diet (3.5% casein) for 28 days. Another group of 122 rats was fed a normal protein diet (26% casein), and a third group of 141 weanlings was fed a commercial lab chow (25% protein from fish, milk, soybean and ground oats). After the feeding period, the rats were fasted overnight and treated with technical grade chlordane by gavage in a range of eight doses. The LD_{50} of chlordane in the low-protein diet group was 137±30 mg/kg bw, in the 26% casein group was 267±44 mg/kg bw and in the commercial diet group was 311 mg/kg bw. Thus, a protein diet protected the rats from chlordane toxicity.

The effects of protein and the quality of protein on heptachlor toxicity in weanling rats was studied in a series of experiments (Webb and Miranda, 1973; Miranda and Webb, 1973, 1974; Miranda et al., 1973; Weatherholtz et al., 1969). When 10% protein diets were fed to weanling rats, heptachlor (administered intraperitoneally) was more toxic when the protein was high quality casein than when the low quality protein, gluten, was used. At 18% protein, this effect was more pronounced (Webb and Miranda, 1973). Rats receiving the gluten diet had reduced body weight, low microsomal protein content and lower activities of heptachlor epoxidase than rats pair-fed the casein diet. Rats maintained on low protein (5% casein) diets were 3 times less susceptible to heptachlor toxicity than were rats pair-fed 20 or 40% casein-containing diets (Weatherholtz et al., 1969). It was suggested that at low protein, metabolism of heptachlor to heptachlor epoxide was inhibited. This hypothesis was tested using an inhibitor (SKF525-A) or an inducer (phenobarbital) of microsomal enzymes (Miranda et al., 1973; Miranda and Webb, 1974). These agents were administered to rats that were maintained on casein or gluten diets before administration of heptachlor or heptachlor epoxide. The results indicated that SKF525-A, rather than protecting the rats from heptachlor, resulted in higher mortality in the gluten-fed rats. Phenobarbital likewise increased the toxicity of heptachlor, as expected, if metabolism to heptachlor epoxide was enhanced. In the casein-fed rats, SKF525-A had little effect on heptachlor toxicity; phenobarbital protected the rats. In similar experiments with heptachlor epoxide, SKF525-A slightly increased the toxicity in gluten-fed rats and greatly increased mortality in casein-fed rats. Phenobarbital completely protected both casein- and gluten-fed rats.

Mechanism of Carcinogenicity

Maslansky and Williams (1981) found that chlordane and heptachlor were negative for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures (see Mutagenicity Section in Chapter V). They concluded that, since the insecticides were not genotoxic, the carcinogenicity involved an epigenetic mechanism, perhaps involving a promotional effect on liver cells already predisposed. They based this conclusion on observations that chlordane and heptachlor induce tumors only in the liver and only in mice that readily develop spontaneous liver lesions. Conversely, Becker and Sell (1979) demonstrated that chlordane induced primary hepatocellular carcinoma in a strain of mice (C57BL/6N) that historically and concurrently did not have spontaneous liver tumors.

Summary

Several studies on the mechanism of neurotoxicity of chlordane and heptachlor were reviewed. Hyde and Falkenberg (1976) described EEG changes in rats during subchronic chlordane exposure. Deprivation of food caused brain potentials indicating lethality, perhaps by causing mobilization of stored chlordane and/or metabolites. Changes in acetylcholine (St. Omer and Ecobichon, 1971) and ammonia and glutamate (St. Omer, 1971) levels in the rat brain were followed with the time course of symptoms of heptachlor toxicity in rats. Maximum acetylcholine levels occurred just before the onset of mild tonic-clonic seizures. Increased ammonia content coincided temporally with mild seizures, while glutamate levels were unaffected by heptachlor treatment.

Yamaguchi et al. (1979, 1980) found that heptachlor epoxide inhibited Ca^{2+}, Mg^{2+}-ATPase in rat brain synaptic vesicles and increased the level of Ca^{2+} in the presynaptic region; they suggested that this promoted the release of neurotransmitter. A 4-fold greater concentration of heptachlor epoxide was found in synaptosomes than in the whole brains of rats that developed violent convulsions after a high dose of heptachlor epoxide. Folmar (1978) found that chlordane and heptachlor inhibited rat brain microsomal Na^+, K^+-ATPase.

Shain et al. (1977) concluded that 5α-dihydrotestosterone binding inhibition experiments were inadequate to predict the effects of heptachlor and chlordane on rat ventral prostate homeostasis. Welch et al. (1971) found that chlordane exposure resulted in decreased fertility of mice. Chlordane and heptachlor were found to enhance the *in vitro* metabolism of estrogen and to suppress the uterotropic action of exogenous estrogen *in vivo*.

Enhanced gluconeogenesis was explained by an action of chlordane on the sympathetic nervous system stimulating secretion of adrenalin and ACTH, which in turn enhanced the production of glucocorticoid hormone (Karel and Saxena, 1976). Alternately, Singhal and Kacew (1976) proposed a direct interaction of chlordane, heptachlor and heptachlor epoxide with an adenyl cyclase receptor on the cell membrane, resulting in cyclic AMP synthesis. Cyclic AMP may react with an intracellular receptor, producing a protein kinase that is translocated to the nucleus where transcription is triggered. The newly synthesized messenger RNA would then induce gluconeogenic enzymes. Microsomal enzymes were also observed to be induced by the insecticides (Kinoshita and Kempf, 1970).

Settlemire et al. (1974) and Pardini et al. (1971) described the effects of heptachlor, heptachlor epoxide and chlordane on cellular respiration.

Phenobarbital potentiated the toxicity of chlordane and heptachlor in neonatal rats (Harbison, 1975). Turpentine potentiated the toxicity of heptachlor, but not heptachlor epoxide in adult rats (Sperling and Ewinike, 1969). Chlordane potentiated the toxicity of CCl_4 in rats (Stenger et al., 1975). A high-protein diet protected rats from chlordane toxicity (Boyd and Taylor, 1969). A low-protein and a poor-quality-protein diet protected rats from heptachlor toxicity (Webb and Miranda, 1973; Miranda and Webb, 1974; Miranda et al., 1973; Weatherholtz et al., 1969). Experiments on the effects of an inhibitor and an inducer of microsomal enzymes on the toxicity of heptachlor in the protein diet groups were equivocal.

Maslansky and Williams (1981) suggested that chlordane and heptachlor exert their carcinogenic effects by an epigenetic mechanism.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \underline{\quad\quad} \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the U.S. EPA (1986a) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]
- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]
- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]
- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{(\text{RfD}) \times (\text{Body weight in kg})}{\text{Drinking Water Volume in } \ell/\text{day}} = \_\_\_\_ \text{ mg}/\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{UF}) \times (\_\_\_ \ \ell/\text{day})} = \_\_\_\_ \text{ mg}/\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Chlordane

Noncarcinogenic Effects. There are several reports of accidental or intentional acute oral exposure of humans to chlordane for which a dose or an estimation of a dose were obtained. In one case, a 32-year-old woman who had ingested 104 mg chlordane/kg body weight died 10 days after exposure (Derbes et al., 1955). Another case of human exposure involved an 18-year-old woman who had ingested 32 mg chlordane/kg body weight (Dadey and Kammer, 1953). It was estimated that she retained 10 mg/kg bw after vomiting. She recovered spontaneously. Lensky and Evans (1952) reported a case of a 15-month-old girl, weighing 9 kg, who drank a small amount of a chlordane suspension. The investigators believed that the amount was <100 mg of chlordane (dose <11.1 mg/kg bw). The child developed tremors, ataxia, convulsions, risus sardonicus, ophisthotonos and other CNS disturbances. Among the symptoms of a 4-year-old girl weighing 11 kg who absorbed an estimated dose of $\geq$0.15 mg chlordane/kg bw were clonic convulsions, loss of coordination, hyporeflexia, excitability and sinus tachycardia (Aldrich and Holmes, 1969). In a similar case, a 20-month-old boy weighing 12.7 kg swallowed 74% technical grade chlordane (Curley and Garrettson, 1969). His symptoms included vomiting, convulsions and seizures. The investigators did not calculate a dose. Some of the signs of toxicity observed may have been associated with the petroleum distillate used in the formulation of liquid commercial products of chlordane.

Much of the information on single oral exposures of laboratory animals to chlordane is concerned with lethality. For rats, reported acute oral lethal dose range from 83-430 mg/kg bw depending on the vehicle and whether technical or pure chlordane was administered (Podowski et al., 1979; Ambrose et al., 1953a,b; Gaines, 1960; Boyd and Taylor, 1969). Gak et al. (1976) reported oral LD_{50}s of 390 mg/kg bw for mice and 1720 mg/kg bw for golden hamsters. Male and female neonatal Sprague-Dawley rats were less sensitive to intraperitoneal administration of chlordane (i.p. LD_{50} = 1121 mg/kg bw) than were adult males (i.p. LD_{50} = 343 mg/kg bw) (Harbison, 1975). Signs of acute chlordane poisoning included CNS disturbances such as tremors, convulsions, irritability, hyporeflexia, dyspnea, and ataxia, among others (Lehman, 1951; Gaines, 1960; Boyd and Taylor, 1969; Stohlman et al., 1950; Arnold et al., 1977). Histological examination of fatally poisoned rats revealed nephritis, hepatitis and vascular congestion (Boyd and Taylor, 1969).

However, sublethal doses have also been tested. Ambrose et al. (1953a,b) observed slight histologic changes in the liver (intracytoplasmic bodies at all dose levels) at doses of 6.25-25 mg/kg bw/day by gavage.

These doses are considered adverse-effect levels (AEL). The next higher dose, 50 mg/kg bw/day resulted in death of 2 of 5 animals. This dose represents a frank-effect level (FEL). At exposures of 10-50 mg/kg diet for 14 days in groups of six male Wistar rats, statistically significant microsomal enzyme induction (aniline hydroxylase, aminopyrine demethylase and hexabarbital oxidase) was observed when compared with controls (Den Tonkelaar and Van Esch, 1974). This exposure corresponds to doses of 1.0-5.0 mg/kg bw/day assuming that a young rat consumes food at a level of 10% of its body weight per day. Based on the effects observed these doses might represent NOAELs. These investigators did not look for other effects. The NOEL for enzyme induction was 5 mg/kg diet (0.05 mg/kg bw/day) for 14 days.

Subchronic dietary exposure of mice and rats to technical grade chlordane for 42 days resulted in increased mortality at 160 and 800 mg/kg diet, respectively (NCI, 1977a). These doses represent FELs. Histopathologic examinations of the animals at these dietary levels or below were not performed. Subchronic feeding of male rats with chlordane for 90 days at a dose of 19.5 mg/kg bw/day resulted in decreased mean weekly body weight gains compared with controls and changes in ventral prostate homeostasis (increased androgen receptor site content, decreased prostate protein, RNA and DNA contents) (Shain et al., 1977). This latter dose can be considered an AEL.

Chronic dietary exposure of male and female rats to chlordane at 0-1280 mg/kg diet for 400 days resulted in a variety of adverse changes. Increased mortality was observed in the two higher lose levels. Dietary levels at or above 80 mg/kg diet in both sexes yielded histopathologic liver changes (vacuolization and enlarged nuclei) that were dose-related. Liver weight was increased in female rats of the 10 mg/kg diet group (Ambrose et al., 1953a,b).

In studies designed to assess the carcinogenicity of chlordane in mice (IRDC, 1973a; NCI, 1977a), such effects as increased mean liver weight, decreased mean body weight and increased mortality were observed among treated groups at dietary levels ranging from 25-63.8 mg/kg diet. In addition, the incidences of hepatocellular carcinoma were significantly increased above controls in these studies. At 5 mg/kg diet in the IRDC (1973a) study, the only observed effects were increased mean liver weights and hepatocytomegaly in females.

In a study by Yonemura et al. (1983) F-344 rats (80/sex/group) were fed technical chlordane at dietary levels of 0, 1, 5 or 25 ppm for 130 weeks. Daily dose levels of 0.045, 0.229 and 1.175 mg/kg for the 1, 5 and 25 ppm treatment groups, respectively, were calculated from food consumption and body weight data. No effects were observed for hematology, clinical chemistry and urinalysis endpoints, and no treatment-related effects were reported for body weight and mortality. Hepatocellular necrosis was observed in 3, 13, 11 and 27 males (64/group) in the 0, 1, 5 and 25 ppm groups, respectively. The increased incidence was statistically significant for all treatment groups. Liver adenomas were found in the high-dose males. The only significant effect in females was hepatocellular swelling in the 25 ppm group.

Increased liver-to-body weight ratios were reported for male and female mice fed chlordane for 2 years at 0.76 ppm (0.09 mg/kg/day), the lowest dose administered (Inui et al., 1983). Liver necrosis was observed at 0.43 and 1.1 mg/kg/day for males only.

Chlordane was not carcinogenic to Osborne-Mendel rats in the NCI (1977a) bioassay; however, tremors, clinical signs of toxicity, reduced mean body weight and increased mortality were observed in various male and female groups treated at 120.8-407.0 mg/kg diet for 80 weeks when compared with controls. Ingle (1952) described the dose-related effects of dietary levels of 5-300 mg/kg diet of chlordane in male and female Osborne-Mendel rats treated for 2 years. At 300 mg/kg diet, severe histopathological damage to liver, kidney, heart, adrenals, lung and spleen; increased mortality; tremors; and hemorrhaging were observed. These effects were less pronounced at 150 mg/kg diet. At 30 mg/kg diet, slight liver damage was observed. At 10 mg/kg diet, minor liver damage consisting of an occasional hypertrophic cell and minor bile duct proliferation was the only effect. No effects on food consumption, growth rate, mortality, no signs of toxicity and no histopathologic changes were observed at 5 mg/kg diet. All of the above doses, with the exception of the last one, represent AELs. The last dose represents a NOAEL.

An additional report by Vettorazzi (1975) describes a review by a WHO/FAO scientific panel of an unpublished study in dogs performed by Wazeter (1967). In this study, the dogs were exposed to diets containing chlordane at levels of 0-30 mg/kg diet for 2 years. The review panel determined that an exposure of 3 mg/kg diet was a NOEL and reported that exposure to 15 or 30 mg/kg diet resulted in increased liver weight and histologic changes in the liver. These latter doses are considered AELs. Assuming that a dog consumes 2.5% of the body weight in food per day the dietary level that is a NOEL is equivalent to a dose of 0.075 mg/kg bw/day.

Ingle (1952) found no teratogenic or fetotoxic effects in neonatal rats following _in utero_ exposure to technical grade chlordane from dams that had been mated in the study discussed above. He did observe toxic symptoms in pups nursed by dams exposed to 150 and 300 mg/kg dietary levels. Toxic symptoms also developed in pups not exposed _in utero_, but nursed by dams treated at these levels. Fertility was reduced to ~50% of control mice in female Swiss Webster mice treated intraperitoneally with three weekly doses of 25 mg/kg bw technical chlordane (Welch et al., 1971).

Quantification of Noncarcinogenic Effects.

Derivation of 1-Day and 10-Day HAs -- Satisfactory dose-response data are not available from which a HA can be derived for 1 and 10 days. However, in order to have some guidelines available, a 10-day HA for chlordane may be derived based upon the Ambrose et al. study (1953a,b) in rats. The toxic effects resulting from daily gastric intubation of doses 6.25, 12.5, 25.0, 50.0, 100.0 or 200 mg/kg chlordane in rats for 15 days were histological changes in the liver of the treated animals (at all dose levels) and central nervous system effects at higher dose levels. The minimal histopathological changes such as presence of abnormal intracytoplasmic bodies of various diameters were evident at dose levels of 6.25 mg/kg. Therefore, this dose level may be used in the development of a 10-day HA and a safety factor of 1000 may be applied in the calculation since data are for the animals rather than human. Accepting 6.25 mg/kg as the minimal effect dose, the HA is derived as follows:

1-Day HA = Insufficient data

The 10-day HA for a child can be calculated as follows:

$$\text{10-day HA} = \frac{(6.25\ \text{mg/kg/day})\ (10\ \text{kg})}{(1000)\ (1\ \ell/\text{day})} = 0.0625\ \text{mg}/\ell$$

$$= 0.063\ \text{mg}/\ell$$

where:

6.25 mg/kg/day = minimal effect level

10 kg = body weight for a child

1000 = uncertainty factor -- intra- and interspecies variation and LOAEL

1 ℓ/day = water consumption per day by a child

It should be noted that this HA for short-term exposure is also applicable for a 1-day HA in view of the metabolism studies of Barnett and Dorough (1974) and Tashiro and Matsumura (1977).

Derivation of Longer-term HA -- There are insufficient toxicological data available to calculate a longer-term HA for chlordane.

The National Research Council Report (NRC, 1982), "An Assessment of the Health Risks of Seven Pesticides Used for Termite Control" was considered for the derivation of a longer-term HA for chlordane. However, the review of the limited human studies with long-term exposure did not reveal any consistent or significant detrimental effect that might be considered for HA level for chlordane. Details of these human studies are given below.

Princi and Spurbeck (1951) evaluated 34 persons engaged in the manufacture of insecticides, including chlordane (exposed through skin contact and inhalation for 11-36 months). Physical examinations, chest X-rays, urinary dilution and concentration tests, routine urinalysis, hemoglobin measurements, sedimentation rate, and urinary porphyrin determinations failed to suggest any abnormalities in the men. The authors concluded that no adverse effects were detected in men working in a plant with air concentrations of chlorinated hydrocarbons as high as 10 mg/m^3. Authors did not specify that exposure was exclusive to chlordane and, therefore, this study was considered inappropriate for a longer-term HA for chlordane.

Alvarez and Hyman (1953) reported a clinical and laboratory study of 24 men 21-49 years old who were exposed to chlordane for 2 months to 5 years while working in a plant where it was manufactured. Each man was given a complete examination, including blood chemistry and urine studies. None of the 24 men had evidence of abnormalities in liver, kidneys, skin, nervous system and blood-forming organs. However, the authors had observed in seven men slight fibrotic changes in the apices of the lungs; one person with a diabetic condition and two more with hypertension in chlordane-exposed workers. These observations (even though not attributed to chlordane) and limited numbers of subjects in this study did not justify its consideration for a longer-term HA level.

Assessment of Lifetime Exposure and Derivation of a DWEL. Chlordane is classified in Group B2, probable human carcinogen, according to EPA's weight-of-evidence scheme for the classification of carcinogenic potential.

The study by Wazeter [(1967), as reported by Vettorazzi, 1975] was considered the most appropriate to derive the DWEL. However, the results of the recent chronic rat dietary study by Yonemura et al. (1983) are available for the derivation of the DWEL. In this study, F344 rats were fed technical chlordane at dietary levels of 0, 1, 5 or 25 ppm for 130 weeks. Clinical laboratory studies were performed and organ weights were measured on eight animals/sex/group at weeks 26 and 52, and on all survivors at week 130. Gross and microscopic pathology were performed on all tissues. Daily dose levels of 0.045, 0.229 and 1.175 mg/kg for the 1, 5 and 25 ppm treatment groups, respectively, were calculated from food consumption and body weight data. No effects were observed for hematology, clinical chemistry and urinalysis endpoints, and no treatment-related effects were reported for body weight and mortality. Hepatocellular necrosis was observed in 3, 13, 11 and 27 males (64/group) in the 0, 1, 5 and 25 ppm groups, respectively. The increased incidence was statistically significant for all treatment groups. Liver adenomas were found in the high-dose males. The only significant effect in females was hepatocellular swelling in the 25 ppm group. The LOAEL of 1 ppm diet (0.045 mg/kg/day) was identified based on liver necrosis in male rats. Using this LOAEL, the DWEL is calculated as follows:

Step 1 - RfD Derivation

$$\text{RfD} = \frac{(0.045\ \text{mg/kg/day})}{(100)\ (10)} = 0.000045\ \text{mg/kg/day} = 0.00005\ \text{mg/kg/day}$$

where:

RfD = Reference Dose: estimate of daily exposure to the human population that appears to be without appreciable risk of deleterious noncarcinogenic effects over a lifetime of exposure

0.045 mg/kg/day = LOAEL

100 = uncertainty factor appropriate for use with data from animal studies

10 = uncertainty factor for use of LOAEL instead of NOAEL

Step 2 - DWEL Derivation

$$\text{DWEL} = \frac{(0.00005\ \text{mg/kg/day})\ (70\ \text{kg})}{(2\ \ell/\text{day})} = 0.0017\ \text{mg}/\ell = 0.002\ \text{mg}/\ell = 2\ \mu\text{g}/\ell$$

where:

70 kg = assumed weight of protected individual (adult)

2 ℓ/day = assumed volume of water ingested per day by 70 kg adult

A summary of the data used to calculate the recommended 1-day and 10-day HAs and the DWEL for chlordane is provided in Table VIII-1.

Carcinogenic Effects.

Human Studies --

Case Reports. There were 11 case reports involving CNS effects where the author looked at the toxic effects of chlordane/heptachlor, eight case studies involving blood dyscrasias, and five case studies of neuroblastomas in children with pre-/postnatal exposure to chlordane or heptachlor. The blood dyscrasias in children included four cases of aplastic anemia and one case each of refractory megaloblastic anemia, acute lymphoblastic leukemia, acute stem-cell leukemia, and acute myelomonocytic leukemia.

Epidemiologic Studies. Three epidemiologic studies of workers exposed to chlordane and/or heptachlor have been reported. One of these studies, conducted in chlordane/heptachlor applicators, was considered inadequate in sample size and in duration. However, this study showed

TABLE VIII-1

Summary of Data Used to Derive HAs and DWELs for Chlordane

Criteria	Animal Dose	Duration	Effect	Value of HA or DWEL			Reference
				Child		Adult	
1-day HA				Insufficient data			
10-day HA	rat, 6.25 mg/kg/day	15 days	Intracycloplasmic bodies of various diameters within hepatocytes	0.063 mg/ℓ			Ambrose et al., 1953a,b
Longer-term HA				Insufficient data			
DWEL	rats	130 weeks	LOAEL for liver necrosis in male rats	--		0.002 mg/ℓ*	Yonemura et al., 1983
Increased lifetime cancer risk level for a 70 kg adult		--	Hepatic carcinomas	10^{-4} 2.7 µg/ℓ	10^{-5} 0.27 µg/ℓ	10^{-6} 0.027 µg/ℓ	U.S. EPA, 1980b

*Assuming 100% contribution being from water inadequate in sample size and in duration. However, this study showed increased mortality from bladder cancer (SMR=277, $p<0.05$). A second study showed an increased mortality from lung cancer (SMR=134), but the increase was not statistically significant. The mortality from cerebrovascular disease was statistically significant (SMR=183, $p\leq0.05$). Of the 1043 men involved in the study, only one liver cancer was reported. The third study involved 2141 workers exposed to organochlorine pesticides. One of the four plants involved in pesticide manufacture produced chlordane and one produced heptachlor. The SMR for malignant neoplasms was 69 at the chlordane plant and 91 at the heptachlor plant. There was an excess risk for cancer in various tissues; none was statistically significant. The last two studies were carried out in chlordane/heptachlor manufacturing plants.

All of these studies have several limitations. Neither the quantitative nor length of exposure histories are available for chlordane/heptachlor for the populations studied. They were also exposed to other pesticides and chemicals. Adjustments for these other chemical exposures and other confounding factors, like smoking and alcohol consumption, were not considered in any of these studies. All of the study populations were small. In the pesticide applicator study, individual follow-up was not undertaken and the data were missing on 10.3% of the decedents reported by the Social Security Administration.

Because of these methodological limitations and the limited data, it is difficult to establish either a negative or positive association between chlordane/heptachlor and carcinogenicity. Hence, these studies are considered inadequate epidemiologic evidence.

Animal Studies --

Chlordane. Four chlordane carcinogenesis bioassays in mice have been reported. The strains tested include C57Bl/6N, CD-1, B6C3F1 and ICR. In C57Bl/6N mice fed 25 or 50 ppm for 18 months, hepatocellular carcinomas were observed in 27% (16) of the survivors. This mouse strain rarely develops spontaneous liver lesions. For CD-1 mice fed 5, 25 or 50 ppm for 18 months, liver nodules/hepatocellular carcinomas were observed in the 25 and 50 ppm groups. In B6C3F1 mice fed ~30 and 60 ppm for 80 weeks and then held for 10 weeks, hepatocellular carcinomas were observed in both males and females. For ICR mice fed 1, 5 or 12.5 ppm for 24 months, hepatocellular adenomas and hemangiomas were significantly increased ($p<0.001$) in males receiving 12.5 ppm and nonneoplastic liver lesions were present in males fed 5 ppm and in females fed 5 or 12.5 ppm.

Four chlordane carcinogenesis bioassays in rats have been reported. The strains tested include albino, Osborne-Mendel and Fischer 344. Three of these studies were considered adequate and one was inadequate. In albino rats fed 10, 20, 40, 80, 160, 320, 640 or 1280 ppm for 400 days, there were no treatment-related tumors. In Osborne-Mendel rats fed 5, 10, 30, 150 or 300 ppm for 2 years, hepatic toxicity was noted at 150 and 300 ppm, but no liver tumors were noted. In Osborne-Mendel rats fed 203.5 or 407 ppm (males) and 120.8 or 241.5 ppm (females), respectively, for 80 weeks and held for an additional 29 weeks, no liver tumors were noted, but thyroid tumors were significantly increased. In light of historical data for Osborne-Mendel rats, the thyroid tumors were not considered to be treatment-related. In Fischer 344 rats fed 1, 5 or 25 ppm for 130 weeks, there was a statistically significant increase in hepatocellular adenomas, which was considered by the authors as weak evidence for carcinogenicity in males fed

25 ppm. Hepatocellular swelling was significant in females fed 25 ppm. The hepatocellular adenomas occurred only in males surviving longer than 104 weeks.

Supporting Evidence --

Mutagenicity. The published literature on mutagenicity testing of chlordane and heptachlor/heptachlor epoxide is quite similar, indeed most studies report results on both chemicals. Generally, the results have indicated that these chemicals are not mutagenic in bacteria or in mammalian cells in culture and do not induce DNA repair as measured by unscheduled DNA synthesis in rodent hepatocytes. While dominant lethal tests in mice have been negative for both chemicals, the absence of direct cytogenetic tests in both germinal and somatic cells precludes a conclusion of their potential for causing chromosome aberrations.

Structural Relationship. Three compounds, structurally related to chlordane/heptachlor/heptachlor epoxide, have induced malignant liver tumors in animals. Aldrin, dieldrin and chlorendic acid have produced liver tumors in mice and chlorendic acid has also produced liver tumors in rats.

Carcinogenicity Classification. Based on the accumulated evidence, chlordane is a probable human carcinogen, classified in Group B2 under the EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986b). Animal studies provide sufficient evidence for carcinogenicity: chlordane increased the incidence of liver carcinomas in C57Bl/6N, CD-1 and B6C3F1 mice; liver adenomas and hemangiomas in ICR mice; and liver adenomas in Fischer 344 rats. Epidemiologic studies provide inadequate evidence due to methodology and data limitations.

According to the criteria in the guidelines, the above evidence puts chlordane in Group B2. However, the guidelines allow for the possibility of downgrading the classification from Group B2 to Group C when the only tumor response is that of mouse liver tumors in strains with high background rates, or when warranted by a number of other factors. In the case of chlordane these conditions do not apply, since chlordane caused tumors in C57Bl/6N mice, which do not have a high background rate, and caused tumors in rats as well. Other pertinent evidence includes highly significant tumor responses, up to 77% increased incidence over controls, increased incidence in both males and females, increased incidence at medium and high doses, a dose-related increase in the proportion of malignant tumors, and induction of tumors by structurally related chemicals. In light of these factors, downgrading is clearly not warranted, and chlordane remains in Group B2.

Quantification of Carcinogenic Effects. For some chemicals, several studies in different animal species, strains and sexes at several doses and different routes of exposure may be available. A choice must be made as to which data sets should be used to quantify human risk by low-dose extrapolation. The following procedure was used to make this choice. The animal studies are evaluated qualitatively to assure that only properly conducted studies are used. The tumor incidence data were separated according to organ sites and tumor types. The data sets used in the model are the ones in which the tumor incidence is statistically significantly higher in at least one test dose level as compared with controls and/or where the tumor incidence rate shows a significant trend with respect to dose level. Both biological and statistical considerations have been used to select the most appropriate data sets.

Because humans may be as sensitive as the most sensitive animal species, potency estimates obtained from the most sensitive species tested can be averaged to estimate potency for the general population. Because some subpopulations may be more sensitive than the general population, the potency estimate from the most sensitive sex and strain tested is also presented. This approach is consistent with EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986b).

As previously described, four data sets showed a significant increase in hepatocellular carcinomas in treatment groups compared with controls in mice. These are male and female mice in the IRDC study, and male and female mice in the NCI study. Tables VIII-2 through VIII-6 give tumor incidence data for these studies.

Estimates of carcinogenic potency can be obtained by fitting the linearized multistage model to each data set. Table VIII-7 summarizes five potency estimates obtained in this way.

Five data sets involve chlordane: male and female CD-1 mice, male and female B6C3F1 mice, and male F344 rats. The most sensitive sex and strain tested is male CD-1 mice. From these, the potency is estimated at 4.7 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 4.7 down to 0.25 per mg/kg/day, with a geometric mean of 1.3 per mg/kg/day. This geometric mean from mice is consistent with

TABLE VIII-2

Cancer Data Sheet for Derivation of Potency of Chlordane from Hepatocellular Carcinomas in Female Mice*

Compound:	Analytical grade chlordane
Species, strain, sex:	Mouse, CD-1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	19.5 months
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	2.98 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	0/45
5	0.65	0.052	0/61
25	3.25	0.260	32/50
50	6.50	0.520	26/37

*Source: IRDC, 1973a

TABLE VIII-3

Cancer Data Sheet for Derivation of Potency of Chlordane from Hepatocellular Carcinomas in Male Mice*

Compound:	Analytical grade chlordane
Species, strain, sex:	Mouse, CD-1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	19.5 months
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	4.74 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	3/33
5	0.65	0.052	5/55
25	3.25	0.260	41/52
50	6.50	0.520	32/39

*Source: IRDC, 1973a

TABLE VIII-4

Cancer Data Sheet for Derivation of Potency of Chlordane from Hepatocellular Carcinomas in Male Mice*

Compound:	Technical grade chlordane
Species, strain, sex:	Mouse, B6C3F1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	0.76 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	2/18
29.9	3.9	0.31	16/48
56.2	7.3	0.58	43/49

*Source: NCI, 1977a

TABLE VIII-5

Cancer Data Sheet for Derivation of Potency of Chlordane from Hepatocellular Carcinomas in Female Mice*

Compound:	Technical grade chlordane
Species, strain, sex:	Mouse, B6C3F1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	0.25 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	0/19
30.1	3.9	0.31	3/47
63.8	8.3	0.66	34/49

*Source: NCI, 1977a

TABLE VIII-6

Cancer Data Sheet for Derivation of Potency of Chlordane from Liver Adenomas and Carcinomas in Male Rats*

Compound:	Technical grade chlordane
Species, strain, sex:	Rat, F344, male
Body weight:	0.35 kg
Length of experiment:	130 weeks
Length of exposure:	130 weeks
Tumor site and type:	Liver, adenoma and carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	1.11 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	1/64
1	0.05	0.17	1/64
5	0.25	0.85	4/64
25	1.25	4.25	9/64

*Source: RIASBT, 1983b

TABLE VIII-7

Human Potency Estimates by Chemical

Chemical	Sex, Species, Strain	Tumor Site, Type	Potency (mg/kg/day)$^{-1}$	Reference
Chlordane	male mice CD-1	liver, carcinoma	4.74	IRDC, 1973a
Chlordane	female mice CD-1	liver, carcinoma	2.98	IRDC, 1973a
Chlordane	male mice B6C3F1	liver, carcinoma	0.76	NCI, 1977a
Chlordane	female mice B6C3F1	liver, carcinoma	0.25	NCI, 1977a
Chlordane	male rats F344	liver, adenoma and carcinoma	1.11	RIASBT, 1983b
Heptachlor	male mice C3H	liver, carcinoma	12.4	Davis, 1965/ Reuber
Heptachlor	female mice C3H	liver, carcinoma	14.9	Davis, 1965/ Reuber
Heptachlor	male mice B6C3F1	liver, carcinoma	2.79	NCI, 1977b
Heptachlor	female mice B6C3F1	liver, carcinoma	0.83	NCI, 1977b
Heptachlor epoxide	male mice C3H	liver, carcinoma	27.7	Davis, 1965/ Reuber
Heptachlor epoxide	female mice C3H	liver, carcinoma	36.2	Davis, 1965/ Reuber
Heptachlor epoxide	female mice CD-1	liver, carcinoma	1.04	IRDC, 1973b/ Reuber
Heptachlor epoxide	male mice CD-1	liver, carcinoma	6.48	IRDC, 1973b/ Reuber
Heptachlor epoxide	female rats CFN	liver, carcinoma	5.76	Witherup et al., 1959/Reuber

the potency estimate from rats of 1.1 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 1.3 per mg/kg/day. These estimates supersede the potency of 1.61 per mg/kg/day previously calculated by the U.S. EPA (1980b).

The upper-limit unit risk estimates from the animal data are derived from a linearized multistage nonthreshold extrapolation model which is currently programmed as GLOBAL 83. Justification for its use is presented in EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986b). While recognizing that alternative statistical modelling approaches exist [e.g., One-hit, Weibull, Log-Probit and Logit models, and maximum likelihood estimates], the range of risks described by using any of these modeling approaches has little biological significance unless data can be used to support the selection of one model over another. In the interest of approach consistency and of providing an upper bound estimate for the potential cancer risk, the Agency recommends the use of the linearized multistage model. EPA considers this model and resulting risk estimate to be an upper limit value in the sense that the true risk is unlikely to be higher and may be lower even zero. An established procedure does not yet exist for making "most likely" or "best" estimates of risk within the range of uncertainty derived by the upper and lower limit values.

The unit risk in water, which is the potency expressed in terms of µg/ℓ drinking water concentrations, is used to estimate risk when exposures are expressed as water concentrations. The unit risk in water is the increased cancer risk to a person who throughout life drinks water contaminated with 1 μg/ℓ of a pollutant. With a linear dose-response curve, risks at any concentration can be computed by multiplying the unit risk by the concentration. The unit risk is computed by converting a 1 μg/ℓ concentration to a mg/kg/day dose and then multiplying by the potency. For a 70 kg person who drinks 2 ℓ a day, a water concentration of 1 μg/ℓ is equivalent to a dose of:

$$(1\ \mu g/\ell)\ (10^{-3}\ mg/\mu g)\ (2\ \ell/day)/(70\ kg) = 2.9\times10^{-5}\ mg/kg/day$$

Multiplying this dose by the potencies calculated above gives unit risks of 3.7×10^{-5} per μg/ℓ for the general population. The unit risk in water is 1.3×10^{-4} per μg/ℓ for the general population and 4.3×10^{-4} per μg/ℓ for sensitive subpopulations.

The concentration in water corresponding to an increased lifetime risk level of 10^{-4}, 10^{-5} and 10^{-6} for a 70 kg human who consumes 2 ℓ/day, is calculated to be 2.7, 0.27 and 0.027 μg/ℓ, respectively.

Heptachlor and Heptachlor Epoxide

Noncarcinogenic Effects. Unlike the case of technical chlordane, reports of effects in humans swallowing quantifiable amounts of technical heptachlor were not located in the available literature. As in the case of chlordane, exposure levels in reports of blood dyscrasia associated with inhalation and dermal exposure of humans to heptachlor are not quantifiable.

Much of the information on single oral exposures of laboratory animals to heptachlor and heptachlor epoxide is concerned with lethality. Heptachlor is more toxic to laboratory animals than chlordane. Acute oral LD_{50} values in rats for heptachlor range from 40 mg/kg bw for a commercial formulation (Ben-Dyke et al., 1970) to 162 mg/kg bw for female Sherman rats for technical grade heptachlor (Gaines et al., 1960). Other LD_{50} values range from 70-100 mg/kg bw in mice and hamsters (Gak et al., 1976). As in the case of chlordane, neonatal rats (i.p. LD_{50} = 531 mg/kg bw) are far less sensitive to intraperitoneal injections of heptachlor than adult males (i.p. LD_{50} = 71 mg/kg bw) (Harbison, 1975). For heptachlor epoxide, the acute oral LD_{50} is ~60 mg/kg bw (Sperling and Ewinike, 1969; Podowski et al., 1979; NAS, 1977). Symptoms of acute intoxication include tumors, convulsions, paralysis, hyperexcitability and irritability (Gaines, 1960; Lehman, 1951; Hrdina et al., 1974).

At a single oral dose of 60 mg heptachlor/kg bw or repeated oral doses of 7 and 12 mg heptachlor/kg bw/day for up to 14 days in rats, significantly increased levels of serum GPT and serum aldolase ($p<0.05-0.001$) were associated with moderate to severe histological liver damage (Krampl, 1971). For rats that had been administered these doses for 28 days, however, the serum levels of these enzymes were not statistically significantly different from controls. The degree of liver damage was less severe than that observed at 7 and 14 days. Rats maintained on diets containing 10 mg heptachlor/kg diet for 1-7 days had evidence of liver damage and altered liver function: increased blood urea, increased blood glucose, decreased liver glycogen content, increased acid and alkaline phosphatase levels when compared with controls (Enan et al., 1982). Liver, heart and spleen weights were also above control weights. Dose-related significant induction of liver microsomal enzymes (aniline hydroxylase, aminopyrine demethylase and hexabarbital oxidase), at dietary levels of heptachlor (>99%) of 2-50 mg/kg diet, was observed in rats (Den Tonkelaar and Van Esch, 1974). The no-effect level was <2 mg/kg diet. However, these investigators did not look for definitive adverse effects. Arnold et al. (1977) reported that a single oral dose of 30 mg/kg bw of a 75% heptachlor epoxide and 25% heptachlor mixture resulted in moderate hypoactivity and 25% mortality in mice.

In dietary studies of 42 days duration, no effects on body weight gain, food consumption or mortality were observed in rats at <80 mg/kg technical heptachlor diet levels or in mice at <40 mg/kg diet levels (NCI, 1977b). Other parameters were not examined. The dietary concentrations are equivalent to doses of 8 mg/kg bw for rats and 12 mg/kg bw for mice, and are quite near the dose levels that resulted in liver damage in rats in the shorter term studies of Krampl (1971). Shain et al. (1977) observed decreased mean weekly body weight gain, reduced food consumption and changes in ventral prostate homeostasis of rats receiving 1.29 mg/kg bw/day of heptachlor. This dose is above the 2 mg/kg diet level for 14 days (i.e., 0.2 mg/kg bw/day) that resulted in enzyme induction in rats (Den Tonkelaar and Van Esch, 1974), but similar to a dose of 10 mg/kg of diet (i.e., 1.0 mg/kg bw/day) that caused definitive adverse effects in rats (Enan et al., 1982). Kinoshita and Kempf (1970) reported in a meeting abstract that the no-effect level in rats for enzyme induction for heptachlor and heptachlor epoxide was 1 mg/kg diet for 91 days. Although details of this study were unavailable from the abstract, the results support the low-effect level for enzyme induction of 2 mg/kg diet reported by Den Tonkelaar and Van Esch (1974).

Several long-term dietary studies with heptachlor and/or heptachlor epoxide, which were designed as carcinogenicity studies, provide toxicologic data. In the NCI (1977b) bioassay in mice, no histopathological signs of toxicity were observed at TWA concentrations of heptachlor of 6.1-18 mg/kg diet for 80 weeks. However, abdominal distention and increased mortality were observed in the high dose females. The incidences of hepatocellular carcinomas were also significant for high dose males and females. Heptachlor was not carcinogenic in rats, but vaginal bleeding also developed in both groups of treated females (25.7 and 51.3 mg/kg diet).

At dietary levels of 10 mg/kg of heptachlor or heptachlor epoxide in mice, Reuber (1977a) diagnosed hepatic vein thrombosis and cirrhosis of the liver, as well as carcinomas, from slides of the Davis (1965) study. In the IRDC (1973b) study, reviewed by Epstein (1976), a 75% heptachlor epoxide and 25% heptachlor mixture was fed to mice for 18 months. Females and males had dose-related increased mean liver weights and hepatocytomegaly at 1, 5 and 10 mg/kg diet. Jolley et al. (1966) found dose-related increased mortality in rats fed 5-12.5 mg/kg diet levels of a 75% heptachlor and 25% heptachlor epoxide mixture for 2 years. Witherup et al. (1955) found non-neoplastic lesions in rats at dietary levels $\geq$7.0 mg/kg diet for 110 weeks. At <5.0 mg/kg diet, no lesions were observed. Treated males had dose-related increased liver weights at levels 1.5-10 mg/kg diet. Although this effect is not necessarily adverse, its presence at doses slightly lower than that which caused tumors and the lack of additional histological analysis dictate that this be considered an adverse effect. The 1.5 mg/kg diet level was the lowest concentration of heptacnlor tested in any of the chronic studies, and thus represents the LOAEL.

Dose-related liver weight increases, hepatocytomegaly and hepatic cell vacuolization were observed in rats maintained for 108 weeks on diets containing heptachlor epoxide at 0.5-10 mg/kg diet (Witherup et al., 1959). These effects are considered to be adverse. Thus, the 0.5 mg/kg diet level represents a LOAEL for heptachlor epoxide.

Dogs were administered heptachlor expoxide in a diet containing various dose levels for 2 years (U.S. EPA, 1971 IRDC, unpublished report). A level of 1 ppm in diet caused no adverse effect on parameters measured. Dose-related changes in biochemical values related to liver function and microscopic changes in liver were noted at the higher dose levels (3, 5, 7 and 10 ppm in diet).

No evidence of teratogenicity of heptachlor or heptachlor epoxide was available. Mestitzova (1967) reported a marked reduction in litter size, however, in rats that were given heptachlor in the diet for several generations. It was not clear whether the dose was 6 mg/kg bw/day or 6 mg/kg bw over the duration of the experiment.

Quantification of Noncarcinogenic Effects. No quantifiable human data on heptachlor or heptachlor epoxide were available. Thus, it is not possible to determine if humans are more sensitive to these chemicals than laboratory animals. In addition, studies on the effects of heptachlor in rabbits were not available to determine interspecies differences among animals. Heptachlor and chlordane are structurally similar organochlorine pesticides. Technical and commercial preparations of either are often contaminated by the other.

__Derivation of 1-Day HA__ -- A NOEL or a NOAEL could not be defined for heptachlor epoxide in the 1- to 30-day studies. As reviewed above, however, a level of 10 mg/kg diet of heptachlor for 14 days in rats was the lowest concentration that resulted in definite adverse effects (i.e., evidence of liver damage and altered liver function (Enan et al., 1982). Although Den Tonkelaar and Van Esch (1974) reported effects at lower doses (i.e., altered liver enzymes), these investigators did not look for any additional effects and those observed are not necessarily adverse. The former study is preferred over the latter as the basis for a HA, because the former study offers additional details. A daily transformed dose (d) can be derived from the former study by assuming a young rat ingests food equivalent to 10% of its body weight/day. The 1-day HA would be calculated from this transformed dose using a 1000-fold uncertainty factor that represents two 10-fold factors for both intra- and interspecies variability to the toxicity of this chemical in lieu of chemical specific information, and an additional 10-fold factor because the HA is derived from a LOAEL and not a NOAEL. Thus, a 1-day HA for a child is derived as follows:

$$\text{1-day HA} = \frac{(1.0\ \text{mg/kg bw})\ (10\ \text{kg})}{(1000)\ (1\ \ell)} = 0.010\ \text{mg}/\ell$$

where:

1.0 mg/kg bw = minimal effect level

10 kg = assumed body weight of a child

1000 = uncertainty factor -- inter- and intraspecies variation and LOAEL

1 ℓ = assumed water consumption per day by a child

Derivation of 10-Day HA -- Few studies of 30-90 days duration that were available defined no- or low-effect levels for heptachlor. In one study that was reported in a meeting abstract (Kinoshita and Kempf, 1970), microsomal enzyme induction occurred in rats in a dose-related way at various dietary levels of heptachlor or heptachlor epoxide administered for 91 days. The NOEL for enzyme induction was 1 mg/kg diet, which is similar to the apparent NOEL for enzyme induction from shorter-term toxicity studies of <2 mg/kg of diet (Den Tonkelaar and Van Esch, 1974).

These results suggest that the rats were either developing a tolerance for heptachlor or responding in a similar fashion as in shorter-term toxicity studies. For these reasons, the 10-day HA for a 10 kg child should be equal to the recommended 1-day HA in order to protect from transitory adverse effects on the liver.

For a child: 10-day HA = 0.010 mg/ℓ

Derivation of Longer-term HA -- There are insufficient toxicological data available to calculate a longer-term HA for heptachlor or heptachlor epoxide.

Assessment of Lifetime Exposure and Derivation of a DWEL --

Heptachlor. The Witherup et al. (1955) study is the most appropriate from which to derive the DWEL for heptachlor. Investigators studied the effects of heptachlor on groups of 20 male and 20 female CF rats. The compound was administered at dietary concentrations of 0, 1.5, 3, 5, 7 or 10 ppm (10 mg/kg/dose) of heptachlor. Mortality among test groups was not dose-related. Loss of body weight, decreased food consumption and increased liver weights were seen among treated males. Lesions in the liver were limited to 7 ppm and above and were characteristic of chlorinated hydrocarbons, i.e., hepatocellular swelling, homogeneity of the cytoplasm and peripheral arrangements of the cytoplasmic granules of cells of the central zone of the liver lobules. The NOEL for increased liver-to-body weight for males only was 3 ppm and the LOEL was 5 ppm. [NOTE: A re-analysis of the Witherup et al. (1955) dietary study on the toxicity of heptachor to rats (by the OPP, RfD Work Group) indicated that the NOEL of 3 ppm (0.15 mg/kg/day) for increased liver-to-body weight for male rats was the most appropriate for a lifetime HA for heptachlor.] Using this NOEL, the DWEL is derived as follows:

Step 1 - RfD Derivation

$$\text{RfD} = \frac{(0.15 \text{ mg/kg/day})}{(100)\ (3)} = 0.0005 \text{ mg/kg/day}$$

where:

RfD = Reference Dose: estimate of daily exposure to the human population that appears to be without appreciable risk of deleterious noncarcinogenic effects over a lifetime of exposure

0.15 mg/kg/day (3 ppm) = NOEL

100 = uncertainty factor appropriate for use with animal studies

3 = additional uncertainty factor to compensate for limited observations and because the most sensitive toxicological endpoint may not have been determined

Step 2 - DWEL Derivation

$$DWEL = \frac{(0.0005\ mg/kg/day)\ (70\ kg)}{(2\ \ell/day)} = 0.0175\ mg/\ell\ (\text{rounded to }20\ \mu g/\ell)$$

where:

0.0005 mg/kg/day = RfD

70 kg = weight of protected individual (adult)

2 ℓ/day = assumed volume of water ingested per day by a 70 kg adult

Heptachlor Epoxide. Two studies using dogs are the most appropriate from which to derive the DWEL. In the 60-week dog feeding study (Kettering Labs., 1958) beagle dogs from 23-27 weeks of age were divided into five groups (three females and two males) and were given diets containing 0, 0.5, 2.5, 5 or 7.5 ppm of heptachlor epoxide. Results included liver-to-body weight ratios that were significantly increased in a treatment-related fashion. Effects were noted for both males and females at the 0.5 ppm (0.0125 mg/kg/day) dose level of heptachlor epoxide. No NOEL was determined for the study. In another 2-generation reproduction study using dogs (U.S. EPA, 1971), animals were administered diets containing various dose levels of heptachlor epoxide. The dose levels were 0, 1, 3, 5, 7 or 10 ppm of heptachlor epoxide in the diet. This study was designed to investigate reproduction parameters associated with heptachlor epoxide administration. The OPP and the RfD Work Group considered that the 60-week dog feeding study providing the LOEL of 0.5 ppm (0.0125 mg/kg/day) is the most appropriate for the derivation of the DWEL. Using this LOEL, the DWEL is derived as follows:

Step 1 - RfD Derivation

$$\text{RfD} = \frac{(0.0125\ \text{mg/kg/day})}{(100)\ (10)} = 0.000013\ \text{mg/kg/day}$$

where:

RfD = Risk Reference Dose: estimate of daily exposure to the human population that appears to be without appreciable risk of deleterious noncarcinogenic effects over a lifetime of exposure

0.0125 mg/kg/day = lowest-observed-effect level (LOEL)

100 = uncertainty factor appropriate for use with animal studies

10 = uncertainty factor to compensate for the fact that a NOEL was not attained

Step 2 - DWEL Derivation

$$\text{DWEL} = \frac{(0.000013\ \text{mg/kg/day})\ (70\ \text{kg})}{(2\ \ell/\text{day})} = 0.00044\ \text{mg}/\ell\ (\text{rounded to } 0.4\ \mu\text{g}/\ell)$$

where:

0.000013 mg/kg/day = RfD

70 kg = weight of protected individual (adult)

2 ℓ/day = assumed volume of water ingested per day by 70 kg adult

A summary of the data used to calculate the recommended 1-day, 10-day and longer-term HAs and the lifetime DWEL for heptachlor/heptachlor epoxide is provided in Table VIII-8.

TABLE VIII-8

Summary of Data Used to Derive HAs and DWELs for Heptachlor/Heptachlor Epoxide

Criteria	Animal Dose	Duration	Effect	Value of HA or DWEL		Reference
				Child	Adult	
1-day HA heptachlor	1.0 mg/kg bw/day	14 days rats	Lowest level for evidence of liver damage and altered liver function	10 µg/ℓ (10 µg/day or 1.0 µg/kg bw)		Enan et al, 1962
10-day HA heptachlor	As above for the 1-day HA					
Longer-term HA	Insufficient Data					
Lifetime DWEL for heptachlor	0.15 mg/kg/day	110 weeks rats	lowest level for increased liver weight	NA	20 µg/ℓ (100% RSC)*	Witherup et al., 1955
Lifetime DWEL for heptachlor epoxide	0.0125 mg/kg bw/day	60 weeks dogs	LOAEL	NA	0.4 µg/ℓ (100% RSC)	Kettering Labs., 1958

*RSC: Percent relative source contribution being from water

NA = Not applicable

Carcinogenic Effects.

Human Studies --

Case Reports. There were 11 case reports involving CNS effects where the author looked at the toxic effects of chlordane/heptachlor, eight case studies involving blood dyscrasias, and five case studies of neuroblastomas in children with pre-/postnatal exposure to chlordane or heptachlor. The blood dyscrasias in children included four cases of aplastic anemia and one case each of refractory megaloblastic anemia, acute lymphoblastic leukemia, acute stem-cell leukemia, and acute myelomonocytic leukemia.

Epidemiologic Studies. Three epidemiologic studies of workers exposed to chlordane and/or heptachlor have been reported. One of these studies, conducted in chlordane/heptachlor applicators, was considered inadequate in sample size and in duration. However, this study showed increased mortality from bladder cancer (SMR=277, $p<0.05$). A second study showed an increased mortality from lung cancer (SMR=134), but the increase was not statistically significant. The mortality from cerebrovascular disease was statistically significant (SMR=183, $p\leq0.05$). Of the 1043 men involved in the study, only one liver cancer was reported. The third study involved 2141 workers exposed to organochlorine pesticides. One of the four plants involved in pesticide manufacture produced chlordane and one produced heptachlor. The SMR for malignant neoplasms was 69 at the chlordane plant and 91 at the heptachlor plant. There was an excess risk for cancer in various tissues; none was statistically significant. The last two studies were carried out in chlordane/heptachlor manufacturing plants.

All of these studies have several limitations. Neither the quantitative nor length of exposure histories are available for chlordane/heptachlor for the populations studied. They were also exposed to other pesticides and chemicals. Adjustments for these other chemical exposures and other confounding factors, like smoking and alcohol consumption, were not considered in any of these studies. All of the study populations were small. In the pesticide applicator study, individual follow-up was not undertaken and the data were missing on 10.3% of the decedents reported by the Social Security Administration.

Because of these methodological limitations and the limited data, it is difficult to establish either a negative or positive association between chlordane/heptachlor and carcinogenicity. Hence, these studies are considered inadequate epidemiologic evidence.

Animal Studies -- Three heptachlor/heptachlor epoxide carcinogenesis bioassays in mice have been reported. The strains studied include C3H, B6C3F1 and CD-1 mice. In C3H mice fed 10 ppm of both heptachlor and heptachlor epoxide for 2 years, benign liver tumors/hepatocellular carcinomas were reported in both male and female mice. Hepatocellular carcinomas in treated groups were generally large and frequently multiple tumors, especially in the epoxide group in respect to the controls. For B6C3F1 mice fed technical grade (containing 22% chlordane) at concentrations of 6.1 or 13.8 ppm (males) or 9 or 18 ppm (females), respectively, for 80 weeks and held for an additional 10 weeks, hepatocellular carcinomas were significantly ($p<0.001$) increased in both male and female mice. In CD-1 mice fed a mixture of heptachlor epoxide/heptachlor (75:25) at concentrations of 1, 5 or 10 ppm for 18 months, nodular hyperplasia/hepatocellular carcinomas were noted at 5 and 10 ppm in both male and female mice.

Five heptachlor/heptachlor epoxide carcinogenesis bioassays in rats have been conducted. The strains of rats studied include Wistar, Osborne-Mendel, CD and CFN. In Wistar rats given five doses of 10 mg/kg bw of heptachlor and held for 106-110 weeks, no treatment-related tumors were observed. For Osborne-Mendel rats fed technical grade heptachlor at concentrations of 38.9 or 77.9 (males) or 25.7 or 51.3 (females) ppm for 80 weeks and held for 30 weeks, no liver tumors were noted, although neoplastic nodules were found in both treated and control rats. In CD rats fed a mixture of heptachlor/heptachlor epoxide (75:25) at concentrations of 5, 7.5, 10 or 12.5 ppm for 2 years, no liver tumors were noted, although nonneoplastic lesions were noted in the livers of rats fed 7.5, 10 or 12.5 ppm. In one study using CFN rats fed 1.5, 3, 5, 7 or 10 ppm of heptachlor for 110 weeks, the incidence of liver tumors was not statistically different in treated and control animals. In a second study using CFN rats fed 0.5, 2.5, 5, 7.5 or 10 ppm of heptachlor epoxide for 108 weeks, treatment-related liver carcinomas were noted by several pathologists.

Supporting Evidence --

Mutagenicity. The published literature on mutagenicity testing of chlordane and heptachlor/heptachlor epoxide is quite similar, indeed most studies report results on both chemicals. Generally, the results have indicated that these chemicals are not mutagenic in bacteria or in mammalian cells in culture and do not induce DNA repair as measured by unscheduled DNA synthesis in rodent hepatocytes. While dominant lethal tests in mice have been negative for both chemicals, the absence of direct cytogenetic tests in both germinal and somatic cells precludes a conclusion of their potential for causing chromosome aberrations.

Structural Relationship. Three compounds, structurally related to chlordane/heptachlor/heptachlor epoxide, have induced malignant liver tumors in animals. Aldrin, dieldrin and chlorendic acid have produced liver tumors in mice and chlorendic acid has also produced liver tumors in rats.

Carcinogenicity Classification. Heptachlor/heptachlor epoxide is a probable human carcinogen, classified in Group B2 under the EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986b). Animal studies provide sufficient evidence for carcinogenicity: heptachlor/heptachlor epoxide increased the incidence of liver carcinomas in C3H, CD-1 and B6C3F1 mice and in CFN rats. Epidemiologic studies provide inadequate evidence due to methodology and data limitations.

The guidelines consider this evidence sufficient for Group B2, but they allow downgrading from Group B2 to Group C when the only tumor response is that of mouse liver tumors in strains with high background rates, or when warranted by a number of other factors. The evidence, however, shows highly significant tumor responses, increased incidence in both males and females, increased incidence at medium and high doses, and induction of tumors by structurally related chemicals. In light of these factors, downgrading is clearly not warranted, and heptachlor/heptachlor epoxide remains in Group B2.

Quantification of Carcinogenic Effects. For some chemicals, several studies in different animal species, strains and sexes at several doses and different routes of exposure may be available. A choice must be made as to which data sets should be used to quantify human risk by low-dose extrapolation. The following procedure was used to make this choice. The animal studies are evaluated qualitatively to assure that only properly conducted studies are used. The tumor incidence data were separated according to organ sites and tumor types. The data sets used in the model are the ones in which the tumor incidence is statistically significantly higher in at least one test dose level as compared with controls and/or where the tumor incidence rate shows a significant trend with respect to dose level. Both biological and statistical considerations have been used to select the most appropriate data sets.

Because humans may be as sensitive as the most sensitive animal species, potency estimates obtained from the most sensitive species tested can be averaged to estimate potency for the general population. Because some subpopulations may be more sensitive than the general population, the potency estimate from the most sensitive sex and strain tested is also presented. This approach is consistent with EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986b).

Eight data sets showed significant increases in the incidence of hepatocellular carcinomas in treated groups compared with controls. Tables VIII-9 through VIII-16 present the tumor incidence for these data sets. In rats, a significant increase in hepatocellular carcinomas was diagnosed by Reuber. Incidence data are shown in Table VIII-17. These studies were used to quantify the carcinogenic risk from heptachlor/heptachlor epoxide exposure.

TABLE VIII-9

Cancer Data Sheet for Derivation of Potency of Heptachlor from Hepatocellular Carcinomas in Male Mice*

Compound:	Heptachlor
Species, strain, sex:	Mouse, C3H, male
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	12.4 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0.00	0.000	22/78
10	1.43	0.108	64/87

*Source: Davis, 1965, as diagnosed by Reuber, 1977b

TABLE VIII-10

Cancer Data Sheet for Derivation of Potency of Heptachlor from Hepatocellular Carcinomas in Female Mice*

Compound:	Heptachlor
Species, strain, sex:	Mouse, C3H, female
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	14.9 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0.00	0.000	2/54
10	1.43	0.108	57/78

*Source: Davis, 1965, as diagnosed by Reuber, 1977b

TABLE VIII-11

Cancer Data Sheet for Derivation of Potency of Heptachlor from Hepatocellular Carcinomas in Male Mice*

Compound:	Technical grade heptachlor
Species, strain, sex:	Mouse, B6C3F1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	2.79 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	5/19
6.1	0.79	0.063	11/46
13.8	1.79	0.14	34/47

*Source: NCI, 1977b

TABLE VIII-12

Cancer Data Sheet for Derivation of Potency of Heptachlor from Hepatocellular Carcinomas in Female Mice*

Compound: Technical grade heptachlor
Species, strain, sex: Mouse, B6C3F1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 90 weeks
Length of exposure: 80 weeks
Tumor site and type: Liver, carcinoma
Route, vehicle: Oral, diet
Human potency (q_1*): 0.83 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	2/10
9.0	1.17	0.094	3/47
18.0	2.34	0.18	30/42

*Source: NCI, 1977b

TABLE VIII-13

Cancer Data Sheet for Derivation of Potency of Heptachlor from Hepatocellular Carcinomas in Male Mice*

Compound:	Heptachlor epoxide
Species, strain, sex:	Mouse, C3H, male
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	27.7 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0.00	0.000	22/78
10	1.43	0.108	73/79

*Source: Davis, 1965, as diagnosed by Reuber, 1977b

TABLE VIII-14

Cancer Data Sheet for Derivation of Potency of Heptachlor from Hepatocellular Carcinomas in Female Mice*

Compound:	Heptachlor epoxide
Species, strain, sex:	Mouse, C3H, female
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	36.2 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0.00	0.000	2/54
10	1.43	0.108	77/81

*Source: Davis, 1965, as diagnosed by Reuber, 1977b

TABLE VIII-15

Cancer Data Sheet for Derivation of Potency of Heptachlor Epoxide from Hepatic Carcinomas in Female Mice*

Compound:	25:75 mixture of heptachlor/ heptachlor epoxide
Species, strain, sex:	Mouse, CD-1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	19 months, 3 weeks
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	1.04 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	6/76
1	0.13	0.01	1/70
5	0.65	0.052	6/65
10	1.30	0.10	30/57

*Source: IRDC, 1973b, as reevaluated by Reuber

TABLE VIII-16

Cancer Data Sheet for Derivation of Potency of Heptachlor Epoxide from Hepatic Carcinomas in Male Mice*

Compound:	25.75 mixture of heptachlor/ heptachlor epoxide
Species, strain, sex:	Mouse, CD-1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	19 months, 3 weeks
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	6.48 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	0/62
1	0.13	0.010	2/68
5	0.65	0.052	18/68
10	1.30	0.10	52/80

*Source: IRDC, 1973b, as reevaluated by Reuber

TABLE VIII-17

Cancer Data Sheet for Derivation of Potency of Heptachlor Epoxide from Hepatic Carcinomas in Female Rats*

Compound:	Heptachlor epoxide
Species, strain, sex:	Rat, CFN, female
Body weight:	0.350 kg (assumed)
Length of experiment:	108 weeks
Length of exposure..	108 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1*):	5.76 per mg/kg/day

Experimental Animal Dose (ppm)	Average Animal Dose (mg/kg/day)	Equivalent Human Dose (mg/kg/day)	Tumor Incidence No. Responding/ No. Examined
0	0	0	0/17
0.5	0.025	0.0043	3/22
2.5	0.125	0.021	3/18
5.0	0.250	0.043	7/22
7.5	0.375	0.064	3/21
10.0	0.500	0.085	5/19

*Source: Witherup et al., 1959, as reevaluated by Reuber

Estimates of carcinogenic potency can be obtained by fitting the linearized multistage model to each data set. Table VIII-7 summarizes nine potency estimates obtained in this way.

Four data sets involve heptachlor: male and female C3H mice, and male and female B6C3F1 mice. The most sensitive sex and strain tested is female C3H mice. From these, the potency is estimated at 14.9 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 14.9 down to 0.83 per mg/kg/day, with a geometric mean of 4.5 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 4.5 per mg/kg/day. These estimates supersede the potency of 3.37 per mg/kg/day previously calculated by the U.S. EPA (1980a).

The upper-limit unit risk estimates from the animal data are derived from a linearized multistage nonthreshold extrapolation model which is currently programmed as GLOBAL 83. Justification for its use is presented in EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986b). While recognizing that alternative statistical modelling approaches exist [e.g., One-hit, Weibull, Log-Probit and Logit models, and maximum likelihood estimates], the range of risks described by using any of these modeling approaches has little biological significance unless data can be used to support the selection of one model over another. In the interest of approach consistency and of providing an upper bound estimate for the potential cancer risk, the Agency recommends the use of the linearized multistage model. EPA considers this model and resulting risk estimate to be an upper limit value in the sense that the true risk is unlikely to be higher and may be lower even zero. An established procedure does not yet exist for making "most likely" or "best" estimates of risk within the range of uncertainty derived by the upper and lower limit values.

The unit risk in water is 1.3×10^{-4} per µg/ℓ for the general population. The concentration in water corresponding to an increased lifetime risk level of 10^{-4}, 10^{-5} and 10^{-6} for a 70 kg human who consumes 2 ℓ/day, is calculated to be 7.6, 0.76 and 0.076 µg/ℓ, respectively.

Five data sets involve heptachlor epoxide: male and female C3H mice, male and female CD-1 mice, and female CFN rats. The most sensitive sex and strain tested is female C3H mice. From these the potency is estimated at 36.2 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 36.2 down to 1.0 per mg/kg/day, with a geometric mean of 9.1 per mg/kg/day. This geometric mean from mice is consistent with the potency estimate from rats of 5.8 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 9.1 per mg/kg/day. These estimates supersede the potency of 57.86 per mg/kg/day previously calculated by the U.S. EPA.

The unit risk in water is 2.6×10^{-5} per µg/ℓ for the general population. The concentration in water corresponding to an increased lifetime risk level of 10^{-4}, 10^{-5} and 10^{-6} for a 70 kg human who consumes 2 ℓ/day, is calculated to be 3.8, 0.38 and 0.038 µg/ℓ, respectively.

Existing Guidelines, Recommendations and Standards

On March 6, 1978, the U.S. EPA (43 FR 12372) cancelled registrations of most pesticide products containing chlordane and heptachlor as defined in the notice of Intent to Cancel (39 FR 41298). The exceptions are the "use of heptachlor or chlordane through subsurface ground insertion for termite control and the dipping of roots or tops of nonfood plants."

Water. The U.S. EPA (40 FR 11990) proposed to set Interim Primary Drinking Water Standards for chlordane at 0.003 mg/ℓ and for heptachlor and heptachlor epoxide at 0.0001 mg/ℓ for maximum contaminant levels. In the final U.S. EPA Regulations (40 FR 59566), however, these levels were deleted because the U.S. EPA was involved in the suspension and cancellation proceedings.

The Federal Water Pollution Control Administration (1968) set permissible surface water criteria for public water supplies at 0.003 mg/ℓ for chlordane and 0.018 mg/ℓ for heptachlor and heptachlor epoxide. The criteria for fish and other aquatic life based on LC_{50} of 0.002 mg/ℓ for chlordane and 0.0002 mg/ℓ for heptachlor would be very low; therefore, it is not recommended that these compounds be used near a marine environment. The Water Quality Criteria for farmstead use were 0.003 mg/ℓ for chlordane and 0.018 mg/mℓ for heptachlor and heptachlor epoxide. Odor thresholds for chlordane and heptachlor in water were reported by Sigworth (1965) as 0.0005 and 0.02 mg/ℓ, respectively.

U.S. EPA (1980b) determined 4.6 ng/ℓ for chlordane as the water concentration corresponding to an increased lifetime risk of cancer of 10^{-5}.

This determination was based upon the incidence of hepatocellular carcinoma in male CD-1 mice following chronic dietary exposure as diagnosed by Reuber in his re-evaluation of slides from an IRDC (1973a) study that was reviewed by Epstein (1976) and upon a bioconcentration factor of 14,100 in fish. U.S. EPA (1980a) determined 2.8 ng/ℓ for heptachlor as the water concentration corresponding to an increased lifetime risk of 10^{-5}. This determination was based upon the incidence of hepatocellular carcinoma in male $B6C3F_1$ mice in the NCI (1977b) bioassay.

Food. FAO/WHO (1978) recommended a maximum acceptable daily intake (ADI) value of 1 µg/kg bw for chlordane. A value of 0.5 µg/kg bw was recommended for heptachlor (FAO/WHO, 1972).

The registration for all uses of chlordane and heptachlor/heptachlor epoxide on food crops has been cancelled (43 FR 12372); however, when these insecticides were in use, the tolerances for residues in or on raw agricultural commodities for chlordane (40 CFR 180.122) and heptachlor and heptachlor epoxide (40 CRF 180.104) are given below:

Section 180.122 Chlordane; tolerances for residues.

> A tolerance of 0.3 part per million is established for residues of the insecticide chlordane (1,2,4,5,6,7,8,8-octachloro-2,3-3a,4,7,7a-hexahydro-4,7-methanoindene, containing not more than 1 percent of the intermediate compound hexachlorocyclopentadiene) in or on each of the following raw agricultural commodities: Apples, apricots, beans, beets (with or without tops) or beet greens alone, blackberries, blueberries (huckleberries), boysenberries, broccoli, brussels sprouts, cabbage, carrots, cauliflower, celery, cherries, citrus fruits, collards, corn, cucumbers, dewberries, eggplants, grapes, kale, kohlrabi, lettuce, loganberries, melons, nectarines, okra, onions, papayas, peaches, peanuts, pears, peas, peppers, pineapples, plums (fresh prunes), potatoes, quinces, radishes (with or without tops) or radish tops, raspberries, rutabagas (with or without tops) or rutabaga tops, squash, strawberries, summer squash, sweet potatoes, tomatoes, turnips (with or without tops) or turnip greens, youngberries.

Section 180.104. Heptachlor and heptachlor epoxide; tolerances for residues.

> 0.1 part per million in or on cabbage, lettuce, rutabagas, snap beans.
>
> Zero in or on alfalfa, apples, barley, beets (including sugar beets), blackeyed peas, brussels sprouts, carrots, cauliflower, cherries, clover, corn, cottonseed, cowpeas, grain sorghum (milo), grapes, grass (pasture and range), kohlrabi, lima beans, meat, milk, oats, onions, peaches, peanuts, peas, pineapple, potatoes, radishes, rye, sugarcane, sweet clover, sweet potatoes, tomatoes, turnips (including tops), wheat.

Air. The American Conference of Governmental Industrial Hygienists (ACGIH, 1983) has adopted TWA-TLVs of 0.5 mg/m³ for chlordane and heptachlor in workroom air. Likewise, the U.S. Occupational Safety and Health Administration standard TLV for exposure in the workroom is 0.5 mg/m³ for chlordane and heptachlor (29 CFR 1910.1000). Corresponding values for heptachlor are 0.5 mg/m³ in the Federal Republic of Germany and 0.01 mg/m³ in the USSR (Winell, 1975).

The U.S. National Research Council (NRC, 1982) has recommended an interim guideline for airborne chlordane and heptachlor in military housing of 5 and 2 μg/m³, respectively.

Special Considerations

High Risk Subpopulations. High risk subpopulations have not been identified in the available literature. Cases of blood dyscrasia have been reported in association with human exposure to chlordane and heptachlor, as well as with other pesticides (Infante et al., 1978; Furie and Trubowitz, 1976; Klemmer et al., 1977). Infante et al. (1978) suggested that an idiosyncratic mechanism for susceptible, but not readily identifiable, individuals may be involved.

Groups that may be or might have been more affected by high exposure rather than by intrinsic susceptibility include exterminators, agricultural applicators, lawn sprayers and workers involved in the manufacture of pesticides. High exposure may also result in persons who live in areas of high pesticide use. Breast-fed infants may be exposed to high levels, since heptachlor epoxide, a major metabolite of heptachlor, has been detected in numerous samples of human milk (Kroger, 1972; Ritcey et al., 1972; Savage et al., 1973; Bakken and Seip, 1976; Polishuk et al., 1977b; Jonsson et al., 1977; Strassman and Kutz, 1977). Oxychlordane, a metabolite of chlordane, was also detected in human milk (Strassman and Kutz, 1977). Developing fetuses may be exposed *in utero*. The transplacental transfer of these chemicals is well documented (Curley et al., 1969; Wasserman et al., 1972, 1974; Zavon et al., 1969; Selby et al., 1969; Polishuk et al., 1977a).

Multiple Pollutant Exposures. Pretreatment of adult male rats with turpentine potentiated the toxicity of heptachlor, reducing the oral LD_{50} from 112 to 70 mg/kg bw (Sperling and Ewinike, 1969). The toxicity of heptachlor epoxide was unaffected. Pretreatment of rats with chlordane resulted in enhancement of hepatocellular necrosis induced by CCl_4 (Stenger et al., 1975).

Several cases of mixed exposure with other pesticides were reported to be associated with blood dyscrasias in humans. These include hemolytic anemia associated with exposure to chlordane, heptachlor, dieldrin and toxaphene (Muirhead et al., 1959); and aplastic anemias, pancytopenia, thrombocytopenia, leukopenia and agranulocytosis associated with chlordane and heptachlor, as well as with other pesticides such as lindane and chlorophenothane (Loge, 1965), Dicamba, Diazinon and 2,4-D (Infante et al., 1978).

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